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(54) **Title:** METHOD FOR DIAGNOSING PANCREATIC CANCER

(57) **Abstract:** Objective methods for detecting and diagnosing pancreatic cancer (PNC) are described herein. In one embodiment, the diagnostic method involves determining the expression level of PNC-associated gene that discriminates between PNC cells and normal cells. The present invention further provides methods of screening for therapeutic agents useful in the treatment of pancreatic cancer, methods of treating pancreatic cancer and method of vaccinating a subject against pancreatic cancer.

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DESCRIPTION

METHOD FOR DIAGNOSING PANCREATIC CANCER

PRIORITY INFORMATION

This application claims priority to United States Provisional Application Serial No.60/414,872, filed September 30, 2002, and Serial No.60/450,889, filed February 28, 2003, and which are incorporated herein by reference.

TECHNICAL FIELD

The invention relates to methods of diagnosing pancreatic cancer.

BACKGROUND OF THE INVENTION

Pancreatic cancer has one of the highest mortality rates of any malignancy, and the 5-year-survival rate of patients is 4%. 28000 patients with pancreatic cancer are diagnosed each year, and nearly all patients will die of their disease (1). The poor prognosis of this malignancy is a result of the difficulty of early diagnosis and poor response to current therapeutic methods (1, 2). In particular currently no tumor markers are identified that allow reliable screening at an early, potentially curative stage of the disease.

cDNA microarray technologies have enabled to obtain comprehensive profiles of gene expression in normal and malignant cells, and compare the gene expression in malignant and corresponding normal cells (Okabe et al., Cancer Res 61:2129-37 (2001); Kitahara et al., Cancer Res 61: 3544-9 (2001); Lin et al., Oncogene 21:4120-8 (2002); Hasegawa et al., Cancer Res 62:7012-7 (2002)). This approach enables to disclose the complex nature of cancer cells, and helps to understand the mechanism of carcinogenesis. Identification of genes that are deregulated in tumors can lead to more precise and accurate diagnosis of individual cancers, and to develop novel therapeutic targets (Bienz and Clevers, Cell 103:311-20 (2000)). To disclose mechanisms underlying tumors from a genome-wide point of view, and discover target molecules for diagnosis and development of novel therapeutic drugs, the present inventors have been analyzing the expression profiles of tumor cells using a cDNA

microarray of 23040 genes (Okabe et al., Cancer Res 61:2129-37 (2001); Kitahara et al., Cancer Res 61:3544-9 (2001); Lin et al., Oncogene 21:4120-8 (2002); Hasegawa et al., Cancer Res 62:7012-7 (2002)).

Studies designed to reveal mechanisms of carcinogenesis have already facilitated identification of molecular targets for anti-tumor agents. For example, inhibitors of farnexyltransferase (FTIs) which were originally developed to inhibit the growth-signaling pathway related to Ras, whose activation depends on posttranslational farnesylation, has been effective in treating Ras-dependent tumors in animal models (He et al., Cell 99:335-45 (1999)). Clinical trials on human using a combination or anti-cancer drugs and anti-HER2 monoclonal antibody, trastuzumab, have been conducted to antagonize the proto-oncogene receptor HER2/neu; and have been achieving improved clinical response and overall survival of breast-cancer patients (Lin et al., Cancer Res 61:6345-9 (2001)). A tyrosine kinase inhibitor, STI-571, which selectively inactivates bcr-abl fusion proteins, has been developed to treat chronic myelogenous leukemias wherein constitutive activation of bcr-abl tyrosine kinase plays a crucial role in the transformation of leukocytes. Agents of these kinds are designed to suppress oncogenic activity of specific gene products (Fujita et al., Cancer Res 61:7722-6 (2001)). Therefore, gene products commonly up-regulated in cancerous cells may serve as potential targets for developing novel anti-cancer agents.

It has been demonstrated that CD8+ cytotoxic T lymphocytes (CTLs) recognize epitope peptides derived from tumor-associated antigens (TAAs) presented on MHC Class I molecule, and lyse tumor cells. Since the discovery of MAGE family as the first example of TAAs, many other TAAs have been discovered using immunological approaches (Boon, Int J Cancer 54: 177-80 (1993); Boon and van der Bruggen, J Exp Med 183: 725-9 (1996); van der Bruggen et al., Science 254: 1643-7 (1991); Brichard et al., J Exp Med 178: 489-95 (1993); Kawakami et al., J Exp Med 180: 347-52 (1994)). Some of the discovered TAAs are now in the stage of clinical development as targets of immunotherapy. TAAs discovered so far include MAGE (van der Bruggen et al., Science 254: 1643-7 (1991)), gp100 (Kawakami et al., J Exp Med 180: 347-52 (1994)), SART (Shichijo et al., J Exp Med 187: 277-88 (1998)), and NY-ESO-1 (Chen et al., Proc Natl Acad Sci USA 94: 1914-8 (1997)). On the other hand, gene products which had been demonstrated to be specifically overexpressed in tumor cells, have been shown to be recognized as targets inducing cellular immune responses. Such gene products include p53 (Umano et al., Brit J Cancer 84: 1052-7 (2001)), HER2/neu (Tanaka et

al., Brit J Cancer 84: 94-9 (2001)), CEA (Nukaya et al., Int J Cancer 80: 92-7 (1999)), and so on.

In spite of significant progress in basic and clinical research concerning TAAs (Rosenbeg et al., Nature Med 4: 321-7 (1998); Mukherji et al., Proc Natl Acad Sci USA 92: 8078-82 (1995); Hu et al., Cancer Res 56: 2479-83 (1996)), only limited number of candidate TAAs for the treatment of adenocarcinomas, including colorectal cancer, are available. TAAs abundantly expressed in cancer cells, and at the same time which expression is restricted to cancer cells would be promising candidates as immunotherapeutic targets. Further, identification of new TAAs inducing potent and specific antitumor immune responses is expected to encourage clinical use of peptide vaccination strategy in various types of cancer (Boon and can der Bruggen, J Exp Med 183: 725-9 (1996); van der Bruggen et al., Science 254: 1643-7 (1991); Brichard et al., J Exp Med 178: 489-95 (1993); Kawakami et al.,J Exp Med 180: 347-52 (1994); Shichijo et al., J Exp Med 187: 277-88 (1998); Chen et al., Proc Natl Acad Sci USA 94: 1914-8 (1997); Harris, J Natl Cancer Inst 88: 1442-5 (1996); Butterfield et al., Cancer Res 59: 3134-42 (1999); Vissers et al., Cancer Res 59: 5554-9 (1999); van der Burg et al., J Immunol 156: 3308-14 (1996); Tanaka et al., Cancer Res 57: 4465-8 (1997); Fujie et al., Int J Cancer 80: 169-72 (1999); Kikuchi et al., Int J Cancer 81: 459-66 (1999); Oiso et al., Int J Cancer 81: 387-94 (1999)).

It has been repeatedly reported that peptide-stimulated peripheral blood mononuclear cells (PBMCs) from certain healthy donors produce significant levels of IFN-γ in response to the peptide, but rarely exert cytotoxicity against tumor cells in an HLA-A24 or –A0201 restricted manner in ^{51}Cr-release assays (Kawano et al., Cance Res 60: 3550-8 (2000); Nishizaka et al., Cancer Res 60: 4830-7 (2000); Tamura et al., Jpn J Cancer Res 92: 762-7 (2001)). However, both of HLA-A24 and HLA-A0201 are one of the popular HLA alleles in Japanese, as well as Caucasian (Date et al., Tissue Antigens 47: 93-101 (1996); Kondo et al., J Immunol 155: 4307-12 (1995); Kubo et al., J Immunol 152: 3913-24 (1994); Imanishi et al., Proceeding of the eleventh International Hictocompatibility Workshop and Conference Oxford University Press, Oxford, 1065 (1992); Williams et al., Tissue Antigen 49: 129 (1997)). Thus, antigenic peptides of carcinomas presented by these HLAs may be especially useful for the treatment of carcinomas among Japanese and Caucasian. Further, it is known that the induction of low-affinity CTL in vitro usually results from the use of peptide at a high concentration, generating a high level of specific peptide/MHC complexes on antigen

presenting cells (APCs), which will effectively activate these CTL (Alexander-Miller et al., Proc Natl Acad Sci USA 93: 4102-7 (1996)).

SUMMARY OF THE INVENTION

The invention is based on the discovery of a pattern of gene expression correlated with pancreatic cancer (PNC). The genes that are differentially expressed in pancreatic cancer are collectively referred to herein as "PNC nucleic acids" or "PNC polynucleotides" and the corresponding encoded polypeptides are referred to as "PNC polypeptides" or "PNC proteins."

Accordingly, the invention features a method of diagnosing or determining a predisposition to pancreatic cancer in a subject by determining an expression level of a PNC-associated gene in a patient derived biological sample, such as tissue sample. By PNC-associated gene is meant a gene that is characterized by an expression level which differs in a cell obtained from a PNC cell compared to a normal cell. A normal cell is one obtained from pancreas tissue. A PNC-associated gene is one or more of PNC 1-605. An alteration, *e.g.*, increase or decrease of the level of expression of the gene compared to a normal control level of the gene indicates that the subject suffers from or is at risk of developing PNC.

By normal control level is meant a level of gene expression detected in a normal, healthy individual or in a population of individuals known not to be suffering from pancreatic cancer. A control level is a single expression pattern derived from a single reference population or from a plurality of expression patterns. For example, the control level can be a database of expression patterns from previously tested cells. A normal individual is one with no clinical symptoms of pancreatic cancer.

An increase in the level of PNC 1-259 detected in a test sample compared to a normal control level indicates the subject (from which the sample was obtained) suffers from or is at risk of developing PNC. In contrast, a decrease in the level of PNC 260-605 detected in a test sample compared to a normal control level indicates said subject suffers from or is at risk of developing PNC.

Alternatively, expression of a panel of PNC-associated genes in the sample is compared to a PNC control level of the same panel of genes. By PNC control level is meant the expression profile of the PNC-associated genes found in a population suffering from PNC.

Gene expression is increased or decreased 10%, 25%, 50% compared to the control level. Alternately, gene expression is increased or decreased 1, 2, 5 or more fold compared to the control level. Expression is determined by detecting hybridization, *e.g.*, on an array, of a PNC-associated gene probe to a gene transcript of the patient-derived tissue sample.

The patient derived tissue sample is any tissue from a test subject, e.g., a patient known to or suspected of having PNC. For example, the tissue contains an epithelial cell. For example, the tissue is an epithelial cell from a pancreatic ductal adenocarcinoma.

The invention also provides a PNC reference expression profile of a gene expression level of two or more of PNC 1-605. Alternatively, the invention provides a PNC reference expression profile of the levels of expression two or more of PNC 1-259 or PNC 260-605.

The invention further provides methods of identifing an agent that inhibits or enhances the expression or activity of a PNC-associated gene, e.g. PNC 1-605 by contacting a test cell expressing a PNC-associated gene with a test agent and determining the expression level of the PNC associated gene. The test cell is a epithelial cell such as an epithelial cell from a pancreatic adenocarcinoma. A decrease of the level compared to a normal control level of the gene indicates that the test agent is an inhibitor of the PNC-associated gene and reduces a symptom of PNC, e.g. PNC 1-259. Alternatively, an increase of the level or activity compared to a normal control level or activity of the gene indicates that said test agent is an enhancer of expression or function of the PNC-associated gene and reduces a symptom of PNC, e.g, PNC 260-605.

The invention also provides a kit with a detection reagent which binds to one or more PNC nucleic acids or which binds to a gene product encoded by the nucleic acid sequences. Also provided is an array of nucleic acids that binds to one or more PNC nucleic acids.

Therapeutic methods include a method of treating or preventing pancreatic cancer in a subject by administering to the subject an antisense composition. The antisense composition reduces the expression of a specific target gene, e.g., the antisense composition contains a nucleotide, which is complementary to a sequence selected from the group consisting of PNC 1-259. Another method includes the steps of administering to a subject an short interfering RNA (siRNA) composition. The siRNA composition reduces the expression of a nucleic acid

selected from the group consisting of PNC 1-259. In yet another method, treatment or prevention of PNC in a subject is carried out by administering to a subject a ribozyme composition. The nucleic acid-specific ribozyme composition reduces the expression of a nucleic acid selected from the group consisting of PNC 1-259. Other therapeutic methods include those in which a subject is administered a compound that increases the expression of PNC 260-605 or activity of a polypeptide encoded by PNC 260-605.

The invention also includes vaccines and vaccination methods. For example, a method of treating or preventing PNC in a subject is carried out by administering to the subject a vaccine containing a polypeptide encoded by a nucleic acid selected from the group consisting of PNC 1-259 or an immunologically active fragment such a polypeptide. An immunologically active fragment is a polypeptide that is shorter in length than the full-length naturally-occurring protein and which induces an immune response. For example, an immunologically active fragment at least 8 residues in length and stimulates an immune cell such as a T cell or a B cell. Immune cell stimulation is measured by detecting cell proliferation, elaboration of cytokines (e.g., IL-2), or production of an antibody.

Alternatively, the present invention provides target molecules for treating or preventing malignant pancreatic cancer. According to the present invention, 76 (PNC 606-681), 168 (PNC 682-849) and 84 (850-933) genes were identified as genes that showed unique altered expression patterns in pancreatic cancer cells with lymph-node metastasis, liver metastasis and early recurrence, respectively. Thus, malignant pancreatic cancer can be treated or prevented via the suppression of the expression or activity of up-regulated genes selected from the group consisting of PNC 606-640 and PNC 682-741. Furthermore, recurrence of pancreatic cancer can be treated or prevented via the suppression of the expression or activity of up-regulated genes selected from the group consisting of PNC 850-893. Moreover, malignant pancreatic cancer can also be treated or prevented through enhancing the expression or activity of down-regulating genes in cancerous cells.

The present invention also provides methods for predicting recurrence of pancreatic cancer. The method comprises the step of measuring the expression level of marker genes selected from the group consisting of PNC 850-879. The marker genes were identified as genes that show unique altered expression patterns in pancreatic cancer cells of patients with recurrence within 12 month after surgery. Therefore, recurrence of the pancreatic cancer in a subject can be predicted by determining whether the expression level

detected in a sample derived from the subject is closer to the mean expression level of early-recurrent cases or late-recurrent cases in reference samples.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

One advantage of the methods described herein is that the disease is identified prior to detection of overt clinical symptoms of pancreatic cancer. Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A is a photograph of a hematoxylin and eosin stained pancreatic cancer (well-differentiated type) before microdissection.1A1 is the same sections after microdissection. 1A2 is a photograph of the microdissected cancer cells captured on the collecting cap.

Figure 1B is a photograph of a hematoxylin and eosin stained pancreatic cancer (scirrhous type) before microdissection. 1B1 is the same sections after microdissection. 1B2 is a photograph of the microdissected cancer cells captured on the collecting cap.

Figure 1C is a photograph of normal pancreas containing greater than 90% acinar cell.

Figure 1D is a photograph of microdissected normall pancreatic ductal epithial cells.

Figure 2 is a photograph of a DNA agarose gel showing expression of representative 12 genes and TUBA examined by semi-quantitative RT-PCR using cDNA prepared from amplified RNA. Lanes 1-12 each show the expression level of the genes in a different PNC patient. Gene symbols are noted for the genes. The last lane shows the expression level of each gene in a normal individual.

Figure 3 Dendrogram of two-dimensional hierarchical clustering analysis using 76 genes selected by a random-permutation test which compared expression profiles of 9 lymph-node positive cases with those of 4 lymph-node negative cases. In the vertical axis, 35 genes were clustered in the upper branch, indicating relatively high levels of expression in lymph-node positive cases.

Figure 4 Dendrogram of two-dimensional hierarchical clustering analysis using 168 genes selected by a random-permutation test which compared expression profiles of 5 liver-metastasis-positive cases with those of 6 negative cases. In the vertical axis, 60 genes were clustered in the upper branch which was more highly expressed in liver-metastasis-positive cases.

Figure 5 (A) Result of a two-dimensional hierarchical clustering analysis using 84 genes selected by a random-permutation test which compared expression profiles of 7 early-recurrent cases (within 12months after surgery) with those of 6 late-recurrent cases (over 12 months after surgery). In the vertical axis, 84 genes were clustered in different branches according to similarity in relative expression ratios. (B) Optimization of the number of discriminating genes. The classification score (CS) was calculated by using the prediction score of early-recurrent case (PSr) and late-recurrent case (PSn) in each gene set, as follows. $CS= (\mu_{PSr} - \mu_{PSn}) / (\sigma_{PSr} + \sigma_{PSn})$. A larger value of CS indicates better separation of the two groups by the predictive-scoring system. (C) Different prediction scores appear when the number of discriminating genes is changed. Red diamonds represent early-recurrent cases; blue diamonds denote late-recurrent cases.

DETAILED DESCRIPTION

Generally pancreatic ductal adenocarcinoma has a characteristic of highly desmoplastic stromal reaction, only a low percentage (about 30%) of cancer cells are contained in the tumor mass. Furthermore, normal pancreatic ductal epithelial cells, which recently considered to be the normal counterpart of the pancreatic adenocarcinoma, occupied only less than 5% of the total population of cells composing the organ 'pancreas' (7, 8). Hence, the gene-expression analysis of PNC compared to normal pancreas by using whole tissue is distorted by the contamination of needless cells such as fibroblast, inflammatory cells, acinar cells, etc., and results in "noisy data". Therefore Laser capture microdissection (LCM),

or Laser microbeam microdissection (LMM), a method for isolating pure cell populations, was used to obtain specific cancer cells and normal epithelial cells (9,10).

The present invention is based in part on the discovery of changes in expression patterns of multiple nucleic acids in epithelial cells from adenocarcinomas of patients with PNC. The differences in gene expression were identified by using a comprehensive cDNA microarray system.

The gene-expression profiles of cancer cells from 18 PNCs were analyzed using cDNA microarray representing 23,040 genes couples with laser microdissection. By comparing expression patterns between cancer cells from diagnostic PNC patients and normal ductal epithelial cells purely selected with Laser Microdisection, 259 genes were identified as commonly up-regulated in PNC cells, and 346 genes were identified as being commonly down-regulated in PNC cells. In addition, selection was made of candidate molecular markers with the potential of detecting cancer-related proteins in serum or sputum of patients, and discovered some potential targets for development of signal-suppressing strategies in human PNC.

The differentially expressed genes identified herein are used for diagnostic purposes as markers of PNC and as gene targets, the expression of which is altered to treat or alleviate a symptom of PNC.

The genes whose expression levels are modulated (*i.e.*, increased or decreased) in PNC patients are summarized in Tables 3-4 and are collectively referred to herein as " PNC-associated genes " , "PNC nucleic acids" or "PNC polynucleotides" and the corresponding encoded polypeptides are referred to as "PNC polypeptides" or "PNC proteins." Unless indicated otherwise, "PNC" is meant to refer to any of the sequences disclosed herein. (*e.g.*, PNC 1-605). The genes have been previously described and are presented along with a database accession number.

By measuring expression of the various genes in a sample of cells, PNC is diagnosed. Similarly, measuring the expression of these genes in response to various agents can identify agents for treating PNC.

The invention involves determining (*e.g.*, measuring) the expression of at least one, and up to all the PNC sequences listed in Tables 3-4. Using sequence information provided by the GeneBank™ database entries for the known sequences the PNC-associated genes are detected and measured using techniques well known to one of ordinary skill in the art. For

example, sequences within the sequence database entries corresponding to PNC sequences, are used to construct probes for detecting PNC RNA sequences in, *e.g.*, northern blot hybridization analysis. Probes include at least 10, 20, 50, 100, 200 nucleotides of a reference sequence. As another example, the sequences can be used to construct primers for specifically amplifying the PNC nucleic acid in, *e.g*, amplification-based detection methods such as reverse-transcription based polymerase chain reaction.

Expression level of one or more of the PNC-associated genes in the test cell population, *e.g.*, a patient derived tissues sample, is then compared to expression levels of the some genes in a reference population. The reference cell population includes one or more cells for which the compared parameter is known, *i.e.*, pancreatic ductal adenocarcinoma cells or normal pancreatic ductal epithelial cells.

Whether or not a pattern of gene expression levels in the test cell population compared to the reference cell population indicates PNC or predisposition thereto depends upon the composition of the reference cell population. For example, if the reference cell population is composed of non-PNC cells, a similar gene expression pattern in the test cell population and reference cell population indicates the test cell population is non-PNC. Conversely, if the reference cell population is made up of PNC cells, a similar gene expression profile between the test cell population and the reference cell population indicates that the test cell population includes PNC cells.

A level of expression of a PNC marker gene in a test cell population is considered altered in levels of expression if its expression level varies from the reference cell population by more than 1.0, 1.5, 2.0, 5.0, 10.0 or more fold from the expression level of the corresponding PNC marker gene in the reference cell population.

Differential gene expression between a test cell population and a reference cell population is normalized to a control nucleic acid, *e.g.* a housekeeping gene. For example, a control nucleic acid is one which is known not to differ depending on the cancerous or non-cancerous state of the cell. Expression levels of the control nucleic acid in the test and reference nucleic acid can be used to normalize signal levels in the compared populations. Control genes include, *e.g.*. β-actin, glyceraldehyde 3- phosphate dehydrogenase or ribosomal protein P1.

The test cell population is compared to multiple reference cell populations. Each of the multiple reference populations may differ in the known parameter. Thus, a test cell

population may be compared to a second reference cell population known to contain, *e.g.*, PNC cells, as well as a second reference population known to contain, *e.g.*, non-PNC cells (normal cells). The test cell is included in a tissue type or cell sample from a subject known to contain, or to be suspected of containing, PNC cells.

The test cell is obtained from a bodily tissue or a bodily fluid, *e.g.*, biological fluid (such as blood or sputum). For example, the test cell is purified from pancreas tissue. Preferably, the test cell population comprises an epithelial cell. The epithelial cell is from tissue known to be or suspected to be a pancreatic ductal adenocarcinoma.

Cells in the reference cell population are derived from a tissue type as similar to test cell. Optionally, the reference cell population is a cell line, *e.g.* a PNC cell line (positive control) or a normal non-PNC cell line (negative control). Alternatively, the control cell population is derived from a database of molecular information derived from cells for which the assayed parameter or condition is known.

The subject is preferably a mammal. The mammal can be, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow.

Expression of the genes disclosed herein is determined at the protein or nucleic acid level using methods known in the art. For example, Northern hybridization analysis using probes which specifically recognize one or more of these nucleic acid sequences can be used to determine gene expression. Alternatively, expression is measured using reverse-transcription-based PCR assays, *e.g.*, using primers specific for the differentially expressed gene sequences. Expression is also determined at the protein level, *i.e.*, by measuring the levels of polypeptides encoded by the gene products described herein, or biological activity thereof. Such methods are well known in the art and include, e.g., immunoassays based on antibodies to proteins encoded by the genes. The biological activity of the proteins encoded by the genes are also well known.

Diagnosing pancreatic cancer

PNC is diagnosed by measuring the level of expression of one or more PNC nucleic acid sequences from a test population of cells, (*i.e.*, a patient derived biological sample). Preferably, the test cell population contains an epithelial cell, e.g., a cell obtained from pancreas tissue. Gene expression is also measured from blood or other bodily fluids such as urine. Other biological samples can be used for measuring the protein level. For example,

the protein level in the blood, serum, or pancreatic juice derived from subject to be diagnosed can be measured by immunoassay or biological assay.

Expression of one or more PNC-associated genes, *e.g.*, PNC 1-605 is determined in the test cell or biological sample and compared to the expression of the normal control level. A normal control level is an expression profile of a PNC-associated gene typically found in a population known not to be suffering from PNC. An increase or a decrease of the level of expression in the patient derived tissue sample of the PNC-associated genes indicates that the subject is suffering from or is at risk of developing PNC. For example, an increase in expression of PNC 1-259 in the test population compared to the normal control level indicates that the subject is suffering from or is at risk of developing PNC. Conversely, a decrease in expression of PNC 260-605 in the test population compared to the normal control level indicates that the subject is suffering from or is at risk of developing PNC.

When one or more of the PNC-associated genes are altered in the test population compared to the normal control level indicates that the subject suffers from or is at risk of developing PNC. For example, at least 1%, 5%, 25%, 50%, 60%, 80%, 90% or more of the panel of PNC-associated genes (PNC 1-259, PNC 260-605, or PNC 1-605) are altered.

Predicting prognosis of PNC

The present invention provides a method for predicting prognosis of PNC in a subject, the method comprising the steps of:

(a) detecting an expression level of one or more marker genes in a specimen collected from a subject to be predicted, wherein the one or more marker genes are selected from the group consisting of PNC 850-866, 894-906; and

(b) comparing the expression level of the one or more marker genes to that of a early recurrence cases and late recurrence cases; and

(c) when the expression level of one or marker genes is close to that of the early recurrence case, determining the subject to be at a risk of having recurrence of PNC and when the expression level of one or marker genes is close to that of the late recurrence case, determining the risk of the subject of having recurrence of PNC to be low.

In the present invention, marker gene(s) for prediction of prognosis of PNC may be at least one gene selected from the group consisting of PNC 850-933 ; 84 genes shown in Table8. The nucleotide sequences of the genes and amino acid sequences encoded thereby are known

in the art. See Table 8 for the Accession Numbers of the genes.

According to the present invention, prediction of prognosis comprises prediction of probability for recurrence of PNC. When recurrence of PNC is observed within 12 month after surgery, the subject is determined to have poor prognosis. In one embodiment, the expression levels of multiple marker genes selected from the group of PNC 850-866, 894-906 can be measured for the prediction. Preferably, the 30 genes consisting of top 17 genes (ARGBP2, CBARA1, EEF1G, LCAT, RPL23A, RPL17, ATP1A1, QARS, BZRP, TUFM, SERPINA4, SCAP, HK1, RPS11, SYNGR2, FLOT2, PSMB4) of up-regulated in late recurrence cases genes and top 13 genes of up-regulated in early recurrence cases genes (MTMR1, HT010, NPD002, YME1L1, CCT6A, HSPD1, TIMM9, GRB14, FLJ10803, LAMP1, MLLT4, CTSB, RALY) of Table 8 are useful for the prediction. In the present method, the specimen is collected from a subject. Preferable specimen include pancreatic tissue derived from patient of pancreatic cancer. Methods for measuring the expression level of marker genes are well-known in the art. For example, DNA array is useful for measuring the expression level of multiple marker genes. According to the present invention, first, the expression level of each marker genes in a specimen is measured and then compared to that of early recurrence cases and late recurrence cases. The expression level of the marker genes of each of the cases can be measured prior to the comparison of the expression level. Then, based on the above comparison, when the expression level of one or marker genes is close to that of the early recurrence case, determining the subject to be at a risk of having recurrence of PNC and when the expression level of one or marker genes is close to that of the late recurrence case, determining the risk of the subject of having recurrence of PNC to be low. In the present invention, the recurrence of PNC can be predicted using prediction score that may be calculated by statistical methods. Methods for calculating prediction score is well-known in the art (T.R. Golub et al., Science 286, 531-7, 1999 ; T.J. MacDonald et al., Nat. Genet, 29, 143-52, 2001). Furthermore, prediction of recurrence using prediction score in the present invention may be also performed according to the method disclosed in the Example.

Identifying Agents that inhibit or enhance PNC-associated gene expression

An agent that inhibits the expression or activity of a PNC-associated gene is identified by contacting a test cell population expressing a PNC-associated up-regulated gene with a test agent and determining the expression level of the PNC-associated gene. A decrease in expression in the presence of the agent compared to the normal control level (or compared to

the level in the absence of the test agent) indicates the agent is an inhibitor of a PNC-associated up-regulated gene and useful to inhibit PNC.

Alternatively, an agent that enhances the expression or activity of a PNC-associated down-regulated gene is identified by contacting a test cell population expressing a PNC-associated gene with a test agent and determining the expression level or activity of the PNC-associated down-regulated gene. An increase of expression or activity compared to a normal control expression level or activity of the PNC-associated gene indicates that the test agent augments expression or activity of the PNC-associated down-regulated gene.

The test cell population is any cell expressing the PNC-associated genes. For example, the test cell population contains an epithelial cell, such as a cell is or derived from pancreas tissue. For example, the test cell is an immortalized cell line derived from an adenocarcinoma cell. Alternatively, the test cell is a cell, which has been transfected with a PNC-associated gene or which has been transfected with a regulatory sequence (*e.g.* promoter sequence) from a PNC-associated gene operably linked to a reporter gene.

Assessing efficacy of treatment of PNC in a subject

The differentially expressed PNC-associated gene identified herein also allow for the course of treatment of PNC to be monitored. In this method, a test cell population is provided from a subject undergoing treatment for PNC. If desired, test cell populations are obtained from the subject at various time points before, during, or after treatment. Expression of one or more of the PNC-associated gene, in the cell population is then determined and compared to a reference cell population which includes cells whose PNC state is known. The reference cells have not been exposed to the treatment.

If the reference cell population contains no PNC cells, a similarity in expression between PNC-associated gene in the test cell population and the reference cell population indicates that the treatment is efficacious. However, a difference in expression between PNC-associated gene in the test population and a normal control reference cell population indicates a less favorable clinical outcome or prognosis.

By "efficacious" is meant that the treatment leads to a reduction in expression of a pathologically up-regulated gene, increase in expression of a pathologically down-regulated gene or a decrease in size, prevalence, or metastatic potential of pancreatic ductal adenocarcinoma in a subject. When treatment is applied prophylactically, "efficacious" means that the treatment retards or prevents a pancreatic tumor from forming or retards,

prevents, or alleviates a symptom of clinical PNC. Assessment of pancreatic tumors is made using standard clinical protocols.

Efficaciousness is determined in association with any known method for diagnosing or treating PNC. PNC is diagnosed for example, by identifying symptomatic anomalies, *e.g.*, weight loss, abdominal pain, back pain, anorexia, nausea, vomiting and generalized malaise, weakness, and jaundice.

Selecting a therapeutic agent for treating PNC that is appropriate for a particular individual

Differences in the genetic makeup of individuals can result in differences in their relative abilities to metabolize various drugs. An agent that is metabolized in a subject to act as an anti-PNC agent can manifest itself by inducing a change in gene expression pattern in the subject's cells from that characteristic of a cancerous state to a gene expression pattern characteristic of a non-cancerous state. Accordingly, the differentially expressed PNC-associated gene disclosed herein allow for a putative therapeutic or prophylactic inhibitor of PNC to be tested in a test cell population from a selected subject in order to determine if the agent is a suitable inhibitor of PNC in the subject.

To identify an inhibitor of PNC, that is appropriate for a specific subject, a test cell population from the subject is exposed to a therapeutic agent, and the expression of one or more of PNC 1-605 genes is determined.

The test cell population contains a PNC cell expressing a PNC-associated gene. Preferably, the test cell is an epithelial cell. For example a test cell population is incubated in the presence of a candidate agent and the pattern of gene expression of the test sample is measured and compared to one or more reference profiles, *e.g.*, a PNC reference expression profile or a non-PNC reference expression profile.

A decrease in expression of one or more of PNC 1-259 or an increase in expression of one or more of PNC 260-605 in a test cell population relative to a reference cell population containing PNC is indicative that the agent is therapeutic.

The test agent can be any compound or composition. For example, the test agents are immunomodulatory agents.

Screening assays for identifying therapeutic agents

The differentially expressed genes disclosed herein can also be used to identify candidate therapeutic agents for treating PNC. The method is based on screening a candidate

therapeutic agent to determine if it converts an expression profile of PNC 1-605 characteristic of a PNC state to a pattern indicative of a non-PNC state.

In the method, a cell is exposed to a test agent or a combination of test agents (sequentially or consequentially) and the expression of one or more PNC 1-605 in the cell is measured. The expression profile of the PNC-associated gene in the test population is compared to expression level of the PNC-associated gene in a reference cell population that is not exposed to the test agent.

An agent effective in stimulating expression of under-expressed genes, or in suppressing expression of over-expressed genes is deemed to lead to a clinical benefit such compounds are further tested for the ability to prevent pancreatic ductal adenocarcinomal growth in animals or test subjects.

In a further embodiment, the present invention provides methods for screening candidate agents which are potential targets in the treatment of PNC. As discussed in detail above, by controlling the expression levels or activities of marker genes, one can control the onset and progression of PNC. Thus, candidate agents, which are potential targets in the treatment of PNC, can be identified through screenings that use the expression levels and activities of marker genes as indices. In the context of the present invention, such screening may comprise, for example, the following steps:

a) contacting a test compound with a polypeptide encoded by PNC 1-605;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting a compound that binds to the polypeptide.

Alternatively, the screening method of the present invention may comprise the following steps:

a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of PNC 1-605; and

b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of PNC 1-259, or elevates the expression level of one or more marker genes selected from the group consisting of PNC 260-605.

Cells expressing a marker gene include, for example, cell lines established from PNC; such cells can be used for the above screening of the present invention.

Alternatively, the screening method of the present invention may comprise the

following steps:

a) contacting a test compound with a polypeptide encoded by selected from the group consisting of PNC 1-605;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide encoded by PNC 1-259 in comparison with the biological activity detected in the absence of the test compound, or enhances the biological activity of the polypeptide encoded by PNC 260-605 in comparison with the biological activity detected in the absence of the test compound.

A protein required for the screening can be obtained as a recombinant protein using the nucleotide sequence of the marker gene. Based on the information of the marker gene, one skilled in the art can select any biological activity of the protein as an index for screening and a measurement method based on the selected biological activity.

Alternatively, the screening method of the present invention may comprise the following steps:

a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of PNC 1-605

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of PNC 1-259 or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of PNC 260-605, as compared to a control.

Suitable reporter genes and host cells are well known in the art. The reporter construct required for the screening can be prepared by using the transcriptional regulatory region of a marker gene. When the transcriptional regulatory region of a marker gene has been known to those skilled in the art, a reporter construct can be prepared by using the previous sequence information. When the transcriptional regulatory region of a marker gene remains unidentified, a nucleotide segment containing the transcriptional regulatory region can be

isolated from a genome library based on the nucleotide sequence information of the marker gene.

The compound isolated by the screening is a candidate for drugs that inhibit the activity of the protein encoded by marker genes and can be applied to the treatment or prevention of pancreatic cancer.

Moreover, compound in which a part of the structure of the compound inhibiting the activity of proteins encoded by marker genes is converted by addition, deletion and/or replacement are also included in the compounds obtainable by the screening method of the present invention.

When administrating the compound isolated by the method of the invention as a pharmaceutical for humans and other mammals, such as mice, rats, guinea-pigs, rabbits, cats, dogs, sheep, pigs, cattle, monkeys, baboons, and chimpanzees, the isolated compound can be directly administered or can be formulated into a dosage form using known pharmaceutical preparation methods. For example, according to the need, the drugs can be taken orally, as sugar-coated tablets, capsules, elixirs and microcapsules, or non-orally, in the form of injections of sterile solutions or suspensions with water or any other pharmaceutically acceptable liquid. For example, the compounds can be mixed with pharmaceutically acceptable carriers or media, specifically, sterilized water, physiological saline, plant-oils, emulsifiers, suspending agents, surfactants, stabilizers, flavoring agents, excipients, vehicles, preservatives, binders, and such, in a unit dose form required for generally accepted drug implementation. The amount of active ingredients in these preparations makes a suitable dosage within the indicated range acquirable.

Examples of additives that can be mixed to tablets and capsules are, binders such as gelatin, corn starch, tragacanth gum and arabic gum; excipients such as crystalline cellulose; swelling agents such as corn starch, gelatin and alginic acid; lubricants such as magnesium stearate; sweeteners such as sucrose, lactose or saccharin; and flavoring agents such as peppermint, Gaultheria adenothrix oil and cherry. When the unit-dose form is a capsule, a liquid carrier, such as an oil, can also be further included in the above ingredients. Sterile composites for injections can be formulated following normal drug implementations using vehicles such as distilled water used for injections.

Physiological saline, glucose, and other isotonic liquids including adjuvants, such as D-sorbitol, D-mannnose, D-mannitol, and sodium chloride, can be used as aqueous solutions

for injections. These can be used in conjunction with suitable solubilizers, such as alcohol, specifically ethanol, polyalcohols such as propylene glycol and polyethylene glycol, non-ionic surfactants, such as Polysorbate 80 (TM) and HCO-50.

Sesame oil or Soy-bean oil can be used as a oleaginous liquid and may be used in conjunction with benzyl benzoate or benzyl alcohol as a solubilizer and may be formulated with a buffer, such as phosphate buffer and sodium acetate buffer; a pain-killer, such as procaine hydrochloride; a stabilizer, such as benzyl alcohol andphenol; and an anti-oxidant. The prepared injection may be filled into a suitable ampule.

Methods well known to one skilled in the art may be used to administer the pharmaceutical composition of the present inevntion to patients, for example as intraarterial, intravenous, or percutaneous injections and also as intranasal, transbronchial, intramuscular or oral administrations. The dosage and method of administration vary according to the body-weight and age of a patient and the administration method; however, one skilled in the art can routinely select a suitable metod of administration. If said compound is encodable by a DNA, the DNA can be inserted into a vector for gene therapy and the vector administered to a patient to perform the therapy. The dosage and method of administration vary according to the body-weight, age, and symptoms of the patient but one skilled in the art can suitably select them.

For example, although the dose of a compound that binds to the protein of the present invention and regulates its activity depends on the symptoms, the dose is about 0.1 mg to about 100 mg per day, preferably about 1.0 mg to about 50 mg per day and more preferably about 1.0 mg to about 20 mg per day, when administered orally to a normal adult (weight 60 kg).

When administering parenterally, in the form of an injection to a normal adult (weight 60 kg), although there are some differences according to the patient, target organ, symptoms and method of administration, it is convenient to intravenously inject a dose of about 0.01 mg to about 30 mg per day, preferably about 0.1 to about 20 mg per day and more preferably about 0.1 to about 10 mg per day. Also, in the case of other animals too, it is possible to administer an amount converted to 60 kgs of body-weight.

Screening assays for identifying therapeutic agents for malignant pancreatic cancer

The present invention provides target molecules for treating or preventing malignant pancreatic cancer. In the present invention, malignant cancer includes cancers having

properties such as follows:

- local invasion;
- aggressive proliferation ; and
- metastasis.

Therefore, according to the present invention, malignant pancreatic cancer includes pancreatic cancer with metastasis. Screening assay for malignant PNC of the present invention can be performed according to the mehtod for PNC described above using marker genes for malignant pancreatic cancer.

In the present invention, marker genes selected from the group consisting of PNC 606-681, and 682-849 are useful for the screening. 76 genes shown in Table 6 (PNC 606-681) were associated with lymph node metastasis. Among the genes, 35 genes (PNC 606-640) were relatively up-regulated and 41 genes (PNC 641-681) were down-regulated in node-positive tumors (Figure 3). In addition, 168 genes (PNC 682-849) showed unique altered expression patterns in pancreatic cells with liver metastasis (Table 7) wherein 60 of the genes(PNC 682-741) were relatively up-regulated (Figure 4). An agent suppressing the activity or expression of these up-regulated genes obtained by the present invention are useful for treating or preventing malignant pancreatic cancer with lymph-node metastasis or liver metastasis. Alternatively, an agent enhancing the activity or expression of the down-regulated genes obtained by the present invention are also useful for treating or preventing malignant pancreatic cancer.

Furthermore, the present invention provides target molecules for treating or preventing recurrence of pancreatic cancer. Herein, recurrence of pancreatic cancer indicates recurrence of cancer in pancreas after surgery. For example, the recurrence of cancer within 12 month after surgery can be predicted by the invention. According to the present invention, early recurrence includes the recurrence within 12 month after surgery, and when no recurrence can be observed within 12 month after surgery in a case, the case is considered to be a pancreatic cancer with "late recurrence". 84 genes (PNC 850-933) shown in Table 8 are useful as the marker genes for the screening of the present invention. Among them, the genes shown in Figure 5A-1 are up-regulated in early recurrence cases(PNC 894-933), and the genes shown in Figure 5A-2 are up-regulated in late recurrence cases(PNC 850-893). Therefore, an agent suppressing the up-regulated genes in early recurrence cases is useful for treating or

preventing recurrence. Alternatively, an agent enhancing the up-regulated genes in late recurrence cases is also useful for treating or preventing recurrence.

Assessing the prognosis of a subject with pancreatic cancer

Also provided is a method of assessing the prognosis of a subject with PNC by comparing the expression of one or more PNC-associated gene in a test cell population to the expression of the genes in a reference cell population derived from patients over a spectrum of disease stages. By comparing gene expression of one or more PNC-associated gene in the test cell population and the reference cell population(s), or by comparing the pattern of gene expression over time in test cell populations derived from the subject, the prognosis of the subject can be assessed.

A decrease in expression of one or more of PNC 260-605 compared to a normal control or an increase of expression of one or more of PNC 1-259 compared to a normal control indicates less favorable prognosis. A similar expression of one or more of PNC 1-605 indicates a more favorable prognosis compared to nomal control indicates a more favorable prognosis for the subject. Preferably, the prognosis of a subject can be assessed by comparing the expression profile of PNC 1-605. The classification score (CS) may be use for the comparing the expression profile.

Kits

The invention also includes a PNC-detection reagent, e.g., a nucleic acid that specifically binds to or identifies one or more PNC nucleic acids such as oligonucleotide sequences, which are complementary to a portion of a PNC nucleic acid or antibodies which bind to proteins encoded by a PNC nucleic acid. The reagents are packaged together in the form of a kit. The reagents are packaged in separate containers, e.g., a nucleic acid or antibody (either bound to a solid matrix or packaged separately with reagents for binding them to the matrix), a control reagent (positive and/or negative), and/or a detectable label. Instructions (e.g., written, tape, VCR, CD-ROM, etc.) for carrying out the assay are included in the kit. The assay format of the kit is a Northern hybridization or a sandwich ELISA known in the art.

For example, PNC detection reagent is immobilized on a solid matrix such as a porous strip to form at least one PNC detection site. The measurement or detection region of the porous strip may include a plurality of sites containing a nucleic acid. A test strip may also

contain sites for negative and/or positive controls. Alternatively, control sites are located on a separate strip from the test strip. Optionally, the different detection sites may contain different amounts of immobilized nucleic acids, *i.e.*, a higher amount in the first detection site and lesser amounts in subsequent sites. Upon the addition of test sample, the number of sites displaying a detectable signal provides a quantitative indication of the amount of PNC present in the sample. The detection sites may be configured in any suitably detectable shape and are typically in the shape of a bar or dot spanning the width of a teststrip.

Alternatively, the kit contains a nucleic acid substrate array comprising one or more nucleic acids. The nucleic acids on the array specifically identify one or more nucleic acid sequences represented by PNC 1-605. The expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by PNC 1-605 are identified by virtue of the level of binding to an array test strip or chip. The substrate array can be on, *e.g.*, a solid substrate, *e.g.*, a "chip" as described in U.S. Patent No.5,744,305.

Arrays and pluralities

The invention also includes a nucleic acid substrate array comprising one or more nucleic acids. The nucleic acids on the array specifically correspond to one or more nucleic acid sequences represented by PNC 1-605. The level of expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by PNC 1-605 are identified by detecting nucleic acid binding to the array.

The invention also includes an isolated plurality (*i.e.*, a mixture if two or more nucleic acids) of nucleic acids. The nucleic acids are in a liquid phase or a solid phase, *e.g.*, immobilized on a solid support such as a nitrocellulose membrane. The plurality includes one or more of the nucleic acids represented by PNC 1-605. In various embodiments, the plurality includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by PNC 1-605.

Methods of inhibiting pancreatic cancer

The invention provides a method for treating or alleviating a symptom of PNC in a subject by decreasing expression or activity of PNC 1-259 or increasing expression or activity of PNC 260-605. Therapeutic compounds are administered prophylactically or therapeutically to subject suffering from or at risk of (or susceptible to) developing PNC. Such subjects are identified using standard clinical methods or by detecting an aberrant level

of expression or activity of PNC 1-605. Therapeutic agents include inhibitors of cell cycle regulation, cell proliferation, and protein kinase activity.

The therapeutic method includes increasing the expression, or function, or both of one or more gene products of genes whose expression is decreased ("under-expressed genes") in a PNC cell relative to normal cells of the same tissue type from which the PNC cells are derived. In these methods, the subject is treated with an effective amount of a compound, which increases the amount of one or more of the under-expressed genes in the subject. Administration can be systemic or local. Therapeutic compounds include a polypeptide product of an under-expressed gene, or a biologically active fragment thereof a nucleic acid encoding an under-expressed gene and having expression control elements permitting expression in the PNC cells; for example an agent which increases the level of expression of such gene endogenous to the PNC cells (i.e., which up-regulates expression of the under-expressed gene or genes). Administration of such compounds counters the effects of aberrantly-under expressed of the gene or genes in the subject's pancreas cells and improves the clinical condition of the subject.

The method also includes decreasing the expression, or function, or both, of one or more gene products of genes whose expression is aberrantly increased ("over-expressed gene") in pancreas cells. Expression is inhibited in any of several ways known in the art. For example, expression is inhibited by administering to the subject a nucleic acid that inhibits, or antagonizes, the expression of the over-expressed gene or genes, e.g., an antisense oligonucleotide or small interfering RNA which disrupts expression of the over-expressed gene or genes.

As noted above, antisense nucleic acids corresponding to the nucleotide sequence of PNC 1-259 can be used to reduce the expression level of the PNC 1-259. Antisense nucleic acids corresponding to PNC 1-259 that are up-regulated in pancreatic cancer are useful for the treatment of pancreatic cancer. Specifically, the antisense nucleic acids of the present invention may act by binding to the PNC 1-259 or mRNAs corresponding thereto, thereby inhibiting the transcription or translation of the genes, promoting the degradation of the mRNAs, and/or inhibiting the expression of proteins encoded by the PNC 1-259, finally inhibiting the function of the proteins. The term "antisense nucleic acids" as used herein encompasses both nucleotides that are entirely complementary to the target sequence and those having a mismatch of one or more nucleotides, so long as the antisense nucleic acids

can specifically hybridize to the target sequences. For example, the antisense nucleic acids of the present invention include polynucleotides that have a homology of at least 70% or higher, preferably at 80% or higher, more preferably 90% or higher, even more preferably 95% or higher over a span of at least 15 continuous nucleotides. Algorithms known in the art can be used to determine the homology.

The antisense nucleic acid derivatives of the present invention act on cells producing the proteins encoded by marker genes by binding to the DNAs or mRNAs encoding the proteins, inhibiting their transcription or translation, promoting the degradation of the mRNAs, and inhibiting the expression of the proteins, thereby resulting in the inhibition of the protein function.

An antisense nucleic acid derivative of the present invention can be made into an external preparation, such as a liniment or a poultice, by mixing with a suitable base material which is inactive against the derivative.

Also, as needed, the derivatives can be formulated into tablets, powders, granules, capsules, liposome capsules, injections, solutions, nose-drops and freeze-drying agents by adding excipients, isotonic agents, solubilizers, stabilizers, preservatives, pain-killers, and such. These can be prepared by following known methods.

The antisense nucleic acids derivative is given to the patient by directly applying onto the ailing site or by injecting into a blood vessel so that it will reach the site of ailment. An antisense-mounting medium can also be used to increase durability and membrane-permeability. Examples are, liposomes, poly-L-lysine, lipids, cholesterol, lipofectin or derivatives of these.

The dosage of the antisense nucleic acid derivative of the present invention can be adjusted suitably according to the patient's condition and used in desired amounts. For example, a dose range of 0.1 to 100 mg/kg, preferably 0.1 to 50 mg/kg can be administered.

The antisense nucleic acids of the invention inhibit the expression of the protein of the invention and is thereby useful for suppressing the biological activity of a protein of the invention. Also, expression-inhibitors, comprising the antisense nucleic acids of the invention, are useful since they can inhibit the biological activity of a protein of the invention.

The antisense nucleic acids of present invention include modified oligonucleotides. For example, thioated nucleotides may be used to confer nuclease resistance to an oligonucleotide.

Also, a siRNA against marker gene can be used to reduce the expression level of the marker gene. By the term "siRNA" is meant a double stranded RNA molecule which prevents translation of a target mRNA. Standard techniques of introducing siRNA into the cell are used, including those in which DNA is a template from which RNA is transcribed. In the context of the present invention, the siRNA comprises a sense nucleic acid sequence and an anti-sense nucleic acid sequence against an up-regulated marker gene, such as PNC 1-259. The siRNA is constructed such that a single transcript has both the sense and complementary antisense sequences from the target gene, e.g., a hairpin.

The method is used to alter the expression in a cell of an up-regulated, e.g., as a result of malignant transformation of the cells. Binding of the siRNA to a transcript corresponding to one of the PNC 1-259 in the target cell results in a reduction in the protein production by the cell. The length of the oligonucleotide is at least 10 nucleotides and may be as long as the naturally-occurring transcript. Preferably, the oligonucleotide is 19-25 nucleotides in length. Most preferably, the oligonucleotide is less than 75, 50, 25 nucleotides in length.

The nucleotide sequence of the siRNAs were designed using an siRNA design computer program available from the Ambion website (http://www.ambion.com/techlib/misc/siRNA_finder.html). The computer program selects nucleotide sequences for siRNA synthesis based on the following protocol.

Selection of siRNA Target Sites:

1. Beginning with the AUG start codon of the object transcript, scan downstream for AA dinucleotide sequences. Record the occurrence of each AA and the 3' adjacent 19 nucleotides as potential siRNA target sites. Tuschl, et al. recommend against designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75 bases) as these may be richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex.
2. Compare the potential target sites to the human genome database and eliminate from consideration any target sequences with significant homology to other coding sequences. The homology search can be performed using BLAST, which can be found on the NCBI server at: www.ncbi.nlm.nih.gov/BLAST/
3. Select qualifying target sequences for synthesis. At Ambion, preferably several target sequences can be selected along the length of the gene to evaluate.

The antisense oligonucleotide or siRNA of the invention inhibit the expression of the polypeptide of the invention and is thereby useful for suppressing the biological activity of the polypeptide of the invention. Also, expression-inhibitors, comprising the antisense oligonucleotide or siRNA of the invention, are useful in the point that they can inhibit the biological activity of the polypeptide of the invention. Therefore, a composition comprising the antisense oligonucleotide or siRNA of the present invention is useful in treating a pancreatic cancer.

Alternatively, function of one or more gene products of the over-expressed genes is inhibited by administering a compound that binds to or otherwise inhibits the function of the gene products. For example, the compound is an antibody which binds to the over-expressed gene product or gene products.

The present invention refers to the use of antibodies, particularly antibodies against a protein encoded by an up-regulated marker gene, or a fragment of the antibody. As used herein, the term "antibody" refers to an immunoglobulin molecule having a specific structure, that interacts (i.e., binds) only with the antigen that was used for synthesizing the antibody (i.e., the up-regulated marker gene product) or with an antigen closely related to it. Furthermore, an antibody may be a fragment of an antibody or a modified antibody, so long as it binds to one or more of the proteins encoded by the marker genes. For instance, the antibody fragment may be Fab, $F(ab')_2$, Fv, or single chain Fv (scFv), in which Fv fragments from H and L chains are ligated by an appropriate linker (Huston J. S. et al. Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883 (1988)). More specifically, an antibody fragment may be generated by treating an antibody with an enzyme, such as papain or pepsin. Alternatively, a gene encoding the antibody fragment may be constructed, inserted into an expression vector, and expressed in an appropriate host cell (see, for example, Co M. S. et al. J. Immunol. 152:2968-2976 (1994); Better M. and Horwitz A. H. Methods Enzymol. 178:476-496 (1989); Pluckthun A. and Skerra A. Methods Enzymol. 178:497-515 (1989); Lamoyi E. Methods Enzymol. 121:652-663 (1986); Rousseaux J. et al. Methods Enzymol. 121:663-669 (1986); Bird R. E. and Walker B. W. Trends Biotechnol. 9:132-137 (1991)).

An antibody may be modified by conjugation with a variety of molecules, such as polyethylene glycol (PEG). The present invention provides such modified antibodies. The modified antibody can be obtained by chemically modifying an antibody. These modification methods are conventional in the field.

Alternatively, an antibody may be obtained as a chimeric antibody, between a variable region derived from a nonhuman antibody and a constant region derived from a human antibody, or as a humanized antibody, comprising the complementarity determining region (CDR) derived from a nonhuman antibody, the frame work region (FR) derived from a human antibody, and the constant region. Such antibodies can be prepared by using known technologies.

Cancer therapies directed at specific molecular alterations that occur in cancer cells have been validated through clinical development and regulatory approval of anti-cancer drugs such as trastuzumab (Herceptin) for the treatment of advanced breast cancer, imatinib methylate (Gleevec) for chronic myeloid leukemia, gefitinib (Iressa) for non-small cell lung cancer (NSCLC), and rituximab (anti-CD20 mAb) for B-cell lymphoma and mantle cell lymphoma (Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. Clin Cancer Res. 2001 Oct;7(10):2958-70. Review.; Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001 Mar 15;344(11):783-92.; Rehwald U, Schulz H, Reiser M, Sieber M, Staak JO, Morschhauser F, Driessen C, Rudiger T, Muller-Hermelink K, Diehl V, Engert A. Treatment of relapsed CD20+ Hodgkin lymphoma with the monoclonal antibody rituximab is effective and well tolerated: results of a phase 2 trial of the German Hodgkin Lymphoma Study Group. Blood. 2003 Jan 15;101(2):420-424.; Fang G, Kim CN, Perkins CL, Ramadevi N, Winton E, Wittmann S and Bhalla KN. (2000). Blood, 96, 2246-2253.). These drugs are clinically effective and better tolerated than traditional anti-cancer agents because they target only transformed cells. Hence, such drugs not only improve survival and quality of life for cancer patients, but also validate the concept of molecularly targeted cancer therapy. Furthermore, targeted drugs can enhance the efficacy of standard chemotherapy when used in combination with it (Gianni L. (2002). Oncology, 63 Suppl 1, 47-56.; Klejman A, Rushen L, Morrione A, Slupianek A and Skorski T. (2002). Oncogene, 21, 5868-5876.). Therefore, future cancer treatments will probably involve combining conventional drugs with target-specific agents aimed at different characteristics of tumor cells such as angiogenesis and invasiveness.

These modulatory methods are performed ex vivo or in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo (e.g., by administering the agent to a subject).

The method involves administering a protein or combination of proteins or a nucleic acid molecule or combination of nucleic acid, molecules as therapy to counteract aberrant expression or activity of the differentially expressed genes.

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity of the genes may be treated with therapeutics that antagonize (i.e., reduce or inhibit) activity of the over-expressed gene or genes. Therapeutics that antagonized activity are administered therapeutically or prophylactically.

Therapeutics that may be utilized include, e.g., (i) a polypeptide, or analogs, derivatives, fragments or homologs thereof of the over-expressed or under-expressed gene or genes; (ii) antibodies to the over-expressed or under-expressed gene or genes; (iii) nucleic acids encoding the over-expressed or under-expressed gene or genes; (iv) antisense nucleic acids or nucleic acids that are "dysfunctional" (i.e., due to a heterologous insertion within the nucleic acids of one or more over-expressed or under-expressed gene or genes); (v) small interfering RNA (siRNA); or (vi) modulators (i.e., inhibitors, agonists and antagonists that alter the interaction between an over/under-expressed polypeptide and its binding partner). The dysfunctional antisense molecules are utilized to "knockout" endogenous function of a polypeptide by homologous recombination (see, e.g., Capecchi, Science 244: 1288-1292 1989). 259

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with therapeutics that increase (i.e., are agonists to) activity. Therapeutics that up-regulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, a polypeptide (or analogs, derivatives, fragments or homologs thereof) or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (e.g., from biopsy tissue) and assaying it in vitro for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of a gene whose expression is altered). Methods that are well-known within the art include, but are not limited to, immunoassays (e.g., by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis,

immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (e.g., Northern assays, dot blots, in situ hybridization, etc.).

Prophylactic administration occurs prior to the manifestation of overt clinical symptoms of disease, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

Therapeutic methods include contacting a cell with an agent that modulates one or more of the activities of the gene products of the differentially expressed genes. An agent that modulates protein activity includes a nucleic acid or a protein, a naturally-occurring cognate ligand of these proteins, a peptide, a peptidomimetic, or other small molecule. For example, the agent stimulates one or more protein activities of one or more of a differentially under-expressed gene.

The present invention also relates to a method of treating or preventing pancreatic cancer in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of PNC 1-259 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide or the fragment thereof. An administration of the polypeptide induces an anti-tumor immunity in a subject. To inducing anti-tumor immunity, a polypeptide encoded by a nucleic acid selected from the group consisting of PNC 1-259 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide is administered. The polypeptide or the immunologically active fragments thereof are useful as vaccines against PNC. In some cases the proteins or fragments thereof may be administered in a form bound to the T cell recepor (TCR) or presented by an antigen presenting cell (APC), such as macrophage, dendritic cell (DC), or B-cells. Due to the strong antigen presenting ability of DC, the use of DC is most preferable among the APCs.

In the present invention, vaccine against PNC refers to a substance that has the function to induce anti-tumor immunity upon inoculation into animals. According to the present invention, polypeptides encoded by PNC 1-259 or fragments thereof were suggested to be HLA-A24 or HLA-A*0201 restricted epitopes peptides that may induce potent and specific immune response against PNC cells expressing PNC 1-259. Thus, the present invention also encompasses method of inducing anti-tumor immunity using the polypeptides. In general, anti-tumor immunity includes immune responses such as follows:

- induction of cytotoxic lymphocytes against tumors,

- induction of antibodies that recognize tumors, and
- induction of anti-tumor cytokine production.

Therefore, when a certain protein induces any one of these immune responses upon inoculation into an animal, the protein is decided to have anti-tumor immunity inducing effect. The induction of the anti-tumor immunity by a protein can be detected by observing in vivo or in vitro the response of the immune system in the host against the protein.

For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. A foreign substance that enters the living body is presented to T cells and B cells by the action of antigen presenting cells (APCs). T cells that respond to the antigen presented by APC in antigen specific manner differentiate into cytotoxic T cells (or cytotoxic T lymphocytes; CTLs) due to stimulation by the antigen, and then proliferate (this is referred to as activation of T cells). Therefore, CTL induction by a certain peptide can be evaluated by presenting the peptide to T cell by APC, and detecting the induction of CTL. Furthermore, APC has the effect of activating CD4+ T cells, CD8+ T cells, macrophages, eosinophils, and NK cells. Since CD4+ T cells and CD8+ T cells are also important in anti-tumor immunity, the anti-tumor immunity inducing action of the peptide can be evaluated using the activation effect of these cells as indicators.

A method for evaluating the inducing action of CTL using dendritic cells (DCs) as APC is well known in the art. DC is a representative APC having the strongest CTL inducing action among APCs. In this method, the test polypeptide is initially contacted with DC, and then this DC is contacted with T cells. Detection of T cells having cytotoxic effects against the cells of interest after the contact with DC shows that the test polypeptide has an activity of inducing the cytotoxic T cells. Activity of CTL against tumors can be detected, for example, using the lysis of ^{51}Cr-labeled tumor cells as the indicator. Alternatively, the method of evaluating the degree of tumor cell damage using ^{3}H-thymidine uptake activity or LDH (lactose dehydrogenase)-release as the indicator is also well known.

Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL is reported that it can be enhanced by culturing PBMC in the presence of GM-CSF and IL-4. Similarly, CTL has been shown to be induced by culturing PBMC in the presence of keyhole limpet hemocyanin (KLH) and IL-7.

The test polypeptides confirmed to possess CTL inducing activity by these methods are polypeptides having DC activation effect and subsequent CTL inducing activity.

Therefore, polypeptides that induce CTL against tumor cells are useful as vaccines against tumors. Furthermore, APC that acquired the ability to induce CTL against tumors by contacting with the polypeptides are useful as vaccines against tumors. Furthermore, CTL that acquired cytotoxicity due to presentation of the polypeptide antigens by APC can be also used as vaccines against tumors. Such therapeutic methods for tumors using anti-tumor immunity due to APC and CTL are referred to as cellular immunotherapy.

Generally, when using a polypeptide for cellular immunotherapy, efficiency of the CTL-induction is known to increase by combining a plurality of polypeptides having different structures and contacting them with DC. Therefore, when stimulating DC with protein fragments, it is advantageous to use a mixture of multiple types of fragments.

Alternatively, the induction of anti-tumor immunity by a polypeptide can be confirmed by observing the induction of antibody production against tumors. For example, when antibodies against a polypeptide are induced in a laboratory animal immunized with the polypeptide, and when growth of tumor cells is suppressed by those antibodies, the polypeptide can be determined to have an ability to induce anti-tumor immunity.

Anti-tumor immunity is induced by administering the vaccine of this invention, and the induction of anti-tumor immunity enables treatment and prevention of PNC. Therapy against cancer or prevention of the onset of cancer includes any of the steps, such as inhibition of the growth of cancerous cells, involution of cancer, and suppression of occurrence of cancer. Decrease in mortality of individuals having cancer, decrease of tumor markers in the blood, alleviation of detectable symptoms accompanying cancer, and such are also included in the therapy or prevention of cancer. Such therapeutic and preventive effects are preferably statistically significant. For example, in observation, at a significance level of 5% or less, wherein the therapeutic or preventive effect of a vaccine against cell proliferative diseases is compared to a control without vaccine administration. For example, Student's t-test, the Mann-Whitney U-test, or ANOVA may be used for statistical analyses.

The above-mentioned protein having immunological activity or a vector encoding the protein may be combined with an adjuvant. An adjuvant refers to a compound that enhances the immune response against the protein when administered together (or successively) with the protein having immunological activity. Examples of adjuvants include cholera toxin, salmonella toxin, alum, and such, but are not limited thereto. Furthermore, the vaccine of this invention may be combined appropriately with a pharmaceutically acceptable

carrier. Examples of such carriers are sterilized water, physiological saline, phosphate buffer, culture fluid, and such. Furthermore, the vaccine may contain as necessary, stabilizers, suspensions, preservatives, surfactants, and such. The vaccine is administered systemically or locally. Vaccine administration may be performed by single administration, or boosted by multiple administrations.

When using APC or CTL as the vaccine of this invention, tumors can be treated or prevented, for example, by the ex vivo method. More specifically, PBMCs of the subject receiving treatment or prevention are collected, the cells are contacted with the polypeptide ex vivo, and following the induction of APC or CTL, the cells may be administered to the subject. APC can be also induced by introducing a vector encoding the polypeptide into PBMCs ex vivo. APC or CTL induced in vitro can be cloned prior to administration. By cloning and growing cells having high activity of damaging target cells, cellular immunotherapy can be performed more effectively. Furthermore, APC and CTL isolated in this manner may be used for cellular immunotherapy not only against individuals from whom the cells are derived, but also against similar types of tumors from other individuals.

Furthermore, a pharmaceutical composition for treating or preventing a cell proliferative disease, such as cancer, comprising a pharmaceutically effective amount of the polypeptide of the present invention is provided. The pharmaceutical composition may be used for raising anti tumor immunity.

Methods for inhibiting development or recurrence of malignant pancreatic cancer

The present invention provides a method for treating or preventing malignant pancreatic cancer, or recurrence of pancreatic cancer by increasing or decreasing the expression or activity of marker genes. According to the present invention, the marker genes that can be used for the treatment or prevention of malignant pancreatic cancer are PNC 606-681 (Table 6) and PNC 682-849 (Table 7). Alternatively, the marker genes for treating or preventing the recurrence are PNC 850-933 (Table 8). 35 genes of the PNC 606-640 (Figure 3) and 60 genes of PNC 682-741 (Figure 4) are up-regulated in the malignant cancer cells and 40 genes of PNC 894-933 are up-regulated in the early recurrence cases. Antisense-nucleotides and siRNAs against any one of the up-regulated marker genes are useful for suppressing the expression of the up-regulated genes. Alternatively, the activity of a protein encoded by any one of the up-regulated marker genes can be inhibited by administering an antibody that binds to the protein. Furthermore, a vaccine against the protein encoded by any

one of the up-regulated marker genes is useful for inducing anti tumor immunity. Moreover, administeration of the down regulated genes or proteins encoded thereby is also effective for treating or preventing malignant pancreatic cancer or the recurrence.

Pharmaceutical compositions for inhibiting PNC, malignant PNC, or recurrence of PNC.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or for administration by inhalation or insufflation. Preferably, administration is intravenous. The formulations are optionally packaged in discrete dosage units.

Pharmaceutical formulations suitable for oral administration include capsules, cachets or tablets, each containing a predetermined amount of the active ingredient. Formulations also include powders, granules or solutions, suspensions or emulsions. The active ingredient is optionally administered as a bolus electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrant or wetting agents. A tablet may be made by compression or molding, optionally with one or more formulational ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be coated according to methods well known in the art. Oral fluid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. The tablets may optionally be formulated so as to provide slow or controlled release of the active ingredient therein. A package of tablets may contain one tablet to be taken on each of the month.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The

formulations may be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Alternatively, the formulations may be presented for continuous infusion. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration include suppositories with standard carriers such as cocoa butter or polyethylene glycol. Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges, which contain the active ingredient in a flavored base such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a base such as gelatin and glycerin or sucrose and acacia. For intra-nasal administration the compounds of the invention may be used as a liquid spray or dispersible powder or in the form of drops. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents.

For administration by inhalation the compounds are conveniently delivered from an insufflator, nebulizer, pressurized packs or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichiorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compounds may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form, in for example, capsules, cartridges, gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflators.

Other formulations include implantable devices and adhesive patches; which release a therapeutic agent.

When desired, the above described formulations, adapted to give sustained release of the active ingredient, may be employed. The pharmaceutical compositions may also contain other active ingredients such as antimicrobial agents, immunosuppressants or preservatives.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

Preferred unit dosage formulations are those containing an effective dose, as recited below, or an appropriate fraction thereof, of the active ingredient.

For each of the aforementioned conditions, the compositions, e.g., polypeptides and organic compounds are administered orally or via injection at a dose of from about 0.1 to about 250 mg/kg per day. The dose range for adult humans is generally from about 5 mg to about 17.5 g/day, preferably about 5 mg to about 10 g/day, and most preferably about 100 mg to about 3 g/day. Tablets or other unit dosage forms of presentation provided in discrete units may conveniently contain an amount which is effective at such dosage or as a multiple of the same, for instance, units containing about 5 mg to about 500 mg, usually from about 100 mg to about 500 mg.

The dose employed will depend upon a number of factors, including the age and sex of the subject, the precise disorder being treated, and its severity. Also the route of administration may vary depending upon the condition and its severity.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims. The following examples illustrate the identification and characterization of genes differentially expressed in PNC cells.

Genome-wide cDNA Microarray Analysis of Gene-Expression Profiles of Pancreatic Cancer Using Cancer and Normal Ductal Epithelial Cells Purely Selected by Laser Microdissection

Tumor markers and targets for therapeutic intervention were identified by analyzing gene-expression profiles using a cDNA microarray representing 23,040 genes. Pancreatic ductal adenocarcinoma that has a characteristic of highly desmoplastic stromal reaction contained a low proportion of cancer cells in the tumor mass. Furthermore, normal duct epithelial cells from which the pancreatic carcinoma originates correspond to a few percent of the pancreas tissue. Therefore, cancer cells were purified from 18 pancreatic cancers by means of laser microbeam microdissection (LMM). Gene expression profiles were examined and compared with those of normal purified pancreatic ductal epithelial cells. These cell populations had been rendered homogenous (more than 95% purified cells). As a result, 259 genes were identified to be commonly up-regulated in pancreatic cancer cells;

among them, the disease correlation and/or function of 64 (including 30 ESTs) genes were not known prior to the invention. The up-regulated genes included ones that were previously reported to be over-expressed in pancreatic cancer, such as interferon-induced transmembrane protein 1 (IFITM1), plasminogen activator, urokinase (PLAU), prostate stem cell antigen (PSCA), S100 calcium binding protein P (S100P), and baculoviral IAP repeat-containing 5 (BIRC5). 346 genes were identified as being commonly down-regulated in pancreatic cancer cells. Of them, 211 genes were functionally characterized and included some tumor suppressor genes such as AXIN1 up-regulated 1(AXUD1), deleted in liver cancer 1 (DLC1), growth arrest and DNA-damage-inducible, beta (GADD45B), p53-inducible p53DINP1 (p53DINP1).

The present gene expression profile represents a highly accurate cancer reference, because a number of limitations of earlier methods were overcome. First, a microarray analysis using clinical samples has been difficult, because of various cellular components are present in the normal as well as cancer tissues. In particular, pancreatic ductal adenocarcinoma that has a characteristic of highly desmoplastic stromal reaction contained a low proportion of cancer cells in the tumor mass. Furthermore, the normal pancreas is mostly constituted from acinar cells and islets that accounted for more than 95% of whole pancreas, and normal duct epithelial cells from which the pancreatic carcinoma originates correspond to a few % of the pancreas. Therefore, the analysis of gene-expression profiles using bulk pancreatic cancer and normal whole pancreatic tissues is significantly influenced by the proportions of cells mixed in the tissues examined; proportional differences of acinar cells, islet cells, fibroblasts, and inflammatory cells may mask the significant increase or decrease of genes that are involved in pancreatic carcinogenesis. Hence, in this study, LMM systems were used to purify cancer and normal epithelial cells from surgical specimens to a high degree of purity (95% or higher). Because it is possible to microdissect even a single cell with LMM, this technology is critical for an accurate microarray analysis of pancreatic cancer specimens. To evaluate the purifity of micordissected pancreatic cancer and normal ductal cells, the expression profile of AMY1A gene which is known to be expressed specifically in acinar cells were analyzed. As a result, the proportion of contaminating acinar cells in the dissected normal pancreatic ductal epithelial cells was estimated to be smaller than 0.29%. In addition to AMY1A, expression levels of other genes that were highly expressed in acinar cells like elastase 1, trypsin 1, and pancreatic lipase were examined. Similar results were

obtained, indicating that the purifity of cell populations by the LMM technique was as high as 99.2%-99.7%.

Second, the quality of extracted RNA from clinical tissue, particularly from pancreas, is one of the most important factors. Pancreas is known to be RNase-rich organ and degradation of RNA occurs very rapidly. In this study, the quality of the extracted RNA from the specimen was examined by visualization of 28S and 18S ribosomal RNAs using denaturing agarose gel electrophoresis. Following electrophoretic analysis, samples in which bands corresponding to two ribosomal RNAs were clearly observed were selected. For example, 18 cases (32%) were selected from the 56 surgically-ressected cases, i.e., many were not included in the analysis due to the poor quality of RNA.

Careful purification of cancer cells as well as normal epitherial ductal cells, subsequent RNA isolation, and cDNA microarray analysis identified 259 genes whose expression was commonly up-regulated (genes which were able to obtain expression data in more than 50% cancer cases and whose expression ratio(Cy5/Cy3 intensity ratio) was more than 5.0 and the genes which were able to calculate in 33 to 50% cases and which expressed the expression ratio of more than 5.0 in all of that cases were also evaluated)

Over 90% of the gene expression profile of pancreatic cancer was different from previous pancreatic cancer expression profiles, because the expression data was obtained by testing highly purified cell populations obtained from patient tissues using laser dissection techniques.

The profiles obtained and described herein represent an improvement over earlier profiles, because they were obtained by analyzing highly purified populations of cancerous cells (pancreatic ductal adenocarcinoma) and compared to a highly purified population of the most relevant normal control, i.e., normal duct epithelial cells. Earlier methods and profiles were hampered by a high percentage of contaminating cells, which reduced the accuracy and reliability of earlier profiles. This present profile is the first one of precise and genome-wide gene expression profiles in large-scale pancreatic cancer. These data identify molecular targets for therapeutic modulation for the treatment of pancreatic cancer and specific novel tumor markers for early and accurate diagnosis of the cancer or a precancerous condition.

EXAMPLE 1: PREPARATION OF TEST SAMPLES

Tissue obtained from diseased tissue (e.g., epithelial cells from PNC) and normal

tissues was evaluated to identify genes which are differently expressed or a disease state, *e.g.*, PNC. The assays were carried out as follows.

Patients, tissue samples, and laser microdissection

Tissue samples of pancreatic cancer (n =18) and normal pancreas (n = 7) were obtained from surgical specimens from patients with informed consent. All pancreatic cancer tissues had histologically confirmed invasive ductal carcinoma. Clinicopathological features of the patients we used in this study are summarized in Table 1. Since almost all pancreatic ductal cells from corresponding normal tissue blocks showed dysplastic changes mostly because of downstream ductal obstruction, ductal cells for only 4 of the 18 pancreatic cancer tissues were suitable to use as normal controls. Hence, additional control ductal cells were obtained from 3 normal pancreas tissues from patients who were operated by cholangiocarcinoma, duodenal leiomyosarcoma, or ampullary tumor. In each case, the specimens were harvested immediately after surgical resection and were embedded in TissueTek OCT medium (Sakura, Tokyo, Japan) before storage at $-80^{\circ}C$. The frozen tissues were cut to 8-μm sections using a cryostat (Sakura, Tokyo, Japan) and then stained with Hematoxylin and Eosin, and check the histological state. Pancreatic carcinoma cells and normal pancreatic ductal epithelial cells were isolated selectively using the EZ cut system with pulsed ultraviolet narrow beam focus laser (SL Microtest GmbH, Germany) in accordance with the manufacturer's protocols. After microdissection, 7 normal cases were mixed to make a "universal control of normal pancreatic ductal epithelial cells ", that was used as a control for all 18 cancer samples.

Table1. Clinocopathological features of the pancreatic cancer patients

Patient No.	Age	Sex	location	pT	pN	M	Stage	Histopathology	Status
1	74	M	pb	1	0	0	I	por	alive
2	56	F	ph	2	0	0	I	pap	alive
3	61	M	ph	1	0	0	I	mod	alive
4	75	M	ph	2	0	0	I	pap	alive
5	unknown	M	ph	3	1	0	III	mod	alive
6	unknown	M	pb	4	0	0	IVA	well	alive
7	77	M	phpb	4	0	0	IVA	mod	dead
8	73	F	ph	4	1	0	IVA	mod	dead
9	75	M	pb	4	1	0	IVA	adenoscc	alive
10	61	M	ph	4	0	0	IVA	mod	alive
11	64	M	ph	4	1	0	IVA	well	dead
12	61	M	ph	4	1	0	IVA	mod	dead

13	65	M	ph	4	1	0	IVA	mod	dead
14	46	M	ph	4	1	0	IVA	mod	dead
15	59	M	ph	4	0	0	IVA	mod=por	alive
16	58	M	ph	4	1	0	IVA	mod	dead
17	74	F	pb	4	1	1	IVB	mod	dead
18	69	F	ph	4	1	1	IVB	mod	dead

Clinical stage was judged according to the UICC TNM classification
location: Tumor location, ph: pancreas head, pb: pancreas body
All patients were Invasive ductal adenocarcinomas, well: Tubular adenocarcinoma well differentiated type
mod: Tubular adenocarcinoma mderately type, por: Tubular adenocarcinoma poorly differentiated type,
pap: Papillary adenocarcinoma, adenoscc: Adenosquamous carcinoma

Isolation of pancreatic cancer cells and normal pancreatic ductal epithelial cells by using LMM

To obtain precise expression profiles of pancreatic cancer cells, LMM was used to purify cancer cells and avoid contamination of non-cancerous cells. In addition, since pancreatic cancer originates from pancreatic ductal cells, pancreatic ductal epithelial cells were used as controls. The great majority of cells in pancreas are acinar cells, it was determined that the use of the entire pancreas was inappropriate for screening genes associated with pancreatic carcinogenesis. As shown in Fig. 1, representative cancer cases (Fig. 1A and 1B), and normal pancreatic duct (Fig. 1C and 1D) were microdissected. Fig. 1A and 1B showed a well-differentiated type and a scirrhous type of invasive ductal adenocarcinoma, and the proportion of cancer cell was about 30% and 10%, respectively. After isolation of pancreatic cancer cells by LMM, we estimated that the proportion of pancreatic cancer cells used in this study was at least 95%.

The proportion of acinar cells contaminated was examined in the microdissected normal pancreatic ductal epithelial cells which used as universal control (Fig1C and 1D). The signal intensity of AMY1A gene was examined that is known to be expressed exclusively in normal acinar cells. The signal intensity of whole pancreatic tissue was investigated in which >90% of the cells are acinar cells, the ratio of the average signal intensity of the pancreatic amylase gene to that of ACTB was approximately 96.7, whereas the ratio of that in microdissected normal pancreatic ductal epithelial cells in this study was calculated approximately 0.28. This result showed the proportion of contaminating acinar cells in the microdissected normal pancreatic ductal epithelial cells was estimated to be 0.29% in average (Fig. 1). Furthermore, the extent of contamination of acinar cells was determined in the microdissected normal pancreatic ductal epithelial cells. Pancreatic amylase gene (AMY1A)

that is expressed exclusively in pancreatic acinar cells was used to evaluate the proportion of the acinar cells in microdissected normal pancreatic ductal epithelial cells. Each intensity was normalized by intensity of β-actin gene (ACTB) as follows;

(Ratio A) the AMY1A /ACTB intensity ratio in whole pancreas (most of the cells correspond to acinar cells) = 96.74

(Ratio B) the AMY1A/ACTB intensity ratio in microdissected normal ductal epithelial cells = 0.28

Contamination percentage (%) ;(Ratio B) /(Ratio A) x 100 =0.29%

Extraction of RNA and T7-based RNA amplification

Total RNAs were extracted from each sample of laser-microdissected cells into 350 μl of RLT lysis buffer (QIAGEN, Hilden, Germany). The extracted RNAs were treated for 30 minutes at room temperature with 30 units of DNase I (Roche, Basel, Switzerland) in the presence of 1 unit of RNase inhibitor (TOYOBO, Osaka, Japan) to remove any contaminating genomic DNA. After inactivation at 70° C for 10 min, the RNAs were purified with an RNeasy Mini Kit (QIAGEN) according to the manufacturer's recommendations. All of the DNase I-treated RNAs were subjected to T7-based RNA amplification as described previously. Two rounds of amplification yielded 50–100μg of aRNA from each sample. A 2.5μg aliquot of aRNA from cancer and normal pancreatic duct epithelial cells was labeled with Cy5-dCTP or Cy3-dCTP, respectively, by a protocol described elsewhere. The hybridization, washing, and scanning were carried out according to the methods described previously (11).

Preparation of the cDNA microarray

A genome-wide cDNA microarray with 23,040 cDNAs selected from the UniGene database (build # 131) of the National Center for Biotechnology Information (NCBI) was constructed. Briefly, the cDNAs were amplified by RT-PCR using poly(A)$^+$ RNA isolated from various human organs as templates; the lengths of the amplicons ranged from 200 to 1,100 bp that did not contain repetitive or poly(A) sequences. The cDNA microarray system was constructed essentially as described previously (11).

Acquisition of data

Signal intensities of Cy3 and Cy5 from the 23,040 spots were quantified and analyzed by substituting backgrounds, using ArrayVision software (Imaging Research, Inc., St. Catharines, Ontario, Canada). Subsequently, the fluorescent intensities of Cy5 (tumor) and Cy3 (control) for each target spot were adjusted so that the mean Cy3/Cy5 ratio of the 52 housekeeping genes was equal to one. Because the data derived from low signal intensities are less reliable, a cut-off value for signal intensities was determined on each slide and excluded genes from further analysis when both Cy3 and Cy5 dyes provided signal intensities lower than the cut-off as described previously (12). For other genes we calculated the Cy5/Cy3 ratio using raw data of each sample.

Semi-Quantitative RT-PCR

The 12 highly up-regulated genes were selected and examined their expression levels by applying the semi-quantitative RT-PCR experiments. A 3-μg aliquot of aRNA from each sample was reversely-transcribed for single-stranded cDNAs using random primer (Roche) and Superscript II (Life Technologies, Inc.). Each cDNA mixture was diluted for subsequent PCR amplification with the same primer sets that were prepared for the target DNA or tubulin, alpha-specific reactions. The primer sequences are listed in Table 2. Expression of tubulin-alpha served as an internal control. PCR reactions were optimized for the number of cycles to ensure product intensity within the linear phase of amplification.

Table 2 Primer sequences for semi-quantitative RT-PCR experiments

PNC Assignment	Accession No.	Symbol	Forward primer	SEQ ID NO.	Reverse primer	SEQ ID NO.
12	AA916826	*APP*	5'-CTGCTGGTCTTCAATTACCAAG-3'	No. 1	5'-CTCATCCCCTTATATTGCCACTT-3'	No. 2
13	L20688	*ARHGDIB*	5'-CTCCCTCTGATCCTCCATCAG-3'	No. 3	5'-TCTTGTTCTCTTGTGTCGTTTACAG-3'	No. 4
15	L24203	*ATDC*	5'-CATTCTCTCTGGCGATGGAGTG-3'	No. 5	5'-ACCAATGGTTTATTCCAAAGGG-3'	No. 6
16	U51478	*ATP1B3*	5'-CAGTGTACAGTCGCCAGATAG-3'	No. 7	5'-TCCTCACATACAGAACTTCTCCAC-3'	No. 8
19	U75285	*BIRC5*	5'-CTCCCTCAGAAAAAGGCAGTG-3'	No. 9	5'-GAAGCTGTAACAATCCACCCTG-3'	No. 10

22	AF068760	*BUB1B*	5'-AGCTAGGCAATCAAGTCTCAC-3'	No. 11	5'-AGGGAAAAGTAGAGACAAATGGG-3'	No. 12
33	AB011536	*CELSR3*	5'-AAGCAGCTTCCTGGGAGATT-3'	No. 13	5'-ACGGAACAATTTACACAGACAGG-3'	No. 14
35	X54941	*CKS1*	5'-ACTATTCGGACAAATACGACGAC-3'	No. 15	5'-CACTGTTTGAATGTGCTGGTAAC-3'	No. 16
36	X54942	*CKS2*	5'-CAAGCAGATCTACTACTCGGACAA-3'	No. 17	5'-CAGTAACCTACTTGCAGTTGCATT-3'	No. 18
48	AA579959	*CYP2S1*	5'-CACCCTGATTCTACCAAATGC-3'	No. 19	5'-CCTTAAGTCACAAGGAACGTCAG-3'	No. 20
54	M91670	*E2-EPF*	5'-TCTGCTCACAGAGATCCACG-3'	No. 21	5'-TTAGAGACAGAGTTGGAGGGAGG-3'	No. 22
56	U32645	*ELF4*	5'-AGAAATGTCAGCCACGGAAAC-3'	No. 23	5'-AAAGGCACTTTAATGCCAACTG-3'	No. 24
57	AF010314	*ENC1*	5'-CGATATAGGCATTTGGTCTCAC-3'	No. 25	5'-TTTCTCTTCATTAGACTTGGCCTCT-3'	No. 26
59	L36645	*EPHA4*	5'-GAAGGCGTGGTCACTAAATGTAA-3'	No. 27	5'-CTTTAATTTCAGAGGGCGAAGAC-3'	No. 28
61	AI627919	*Evi-1*	5'-GCAAGCTTGTGCGATGTTATGT-3'	No. 29	5'-CTCCTCCCATAGTAATGCACTGA-3'	No. 30
63	L16783	*FOXM1*	5'-GATGGATGCAACTGAAGCAGAG-3'	No. 31	5'-GTCCACCTTCGCTTTTATTGAGT-3'	No. 32
73	AA652197	*GW112*	5'-GAAAATCTGATGGCAGTGACAA-3'	No. 33	5'-AAGGTTTCCAACTACTGCACTGA-3'	No. 34
74	J04501	*GYS1*	5'-TGCCCACTGTGAAACCACTAG-3'	No. 35	5'-CATCTCATCTCCGGACACACT-3'	No. 36
77	D16431	*HDGF*	5'-TATCCCAGCTGCCTAGATTC-3'	No. 37	5'-GAGTCTTCCCAAGCATCCTATTT-3'	No. 38
83	M16937	*HOXB7*	5'-GTACCTATAGGAAAGTCTGTC-3'	No. 39	5'-AACACGCGAGTGGTAGGTTTT-3'	No. 40
84	AA495868	*hPAD-colony10*	5'-CACTGAGCCAACTACTGTCACTG-3'	No. 41	5'-CTTCCTACCCACAGCTCTTTCTC-3'	No. 42
102	U63743	*KNSL6*	5'-ACTCTAGGACTTGCATGATTGCC-3'	No. 43	5'-TCCTCTAGGACTCTAGGGAGACA-3'	No. 44
103	U70322	*KPNB2*	5'-TCTTGGAGACTATAAGGGAGCC-3'	No. 45	5'-TTTTGCTTCTTCACATCCACTG-3'	No. 46
115	X57766	*MMP11*	5'-GCACTGAAGCAAGGGTGCTG-3'	No. 47	5'-GACAGGATTGAGGTATGTTGCAG-3'	No. 48

120	X13293	*MYBL2*	5'-TCCTGAGGTGTTGAGGGTGTC-3'	No. 49	5'-ATCCTAAGCAGGGTCTGAGATG-3'	No. 50
125	X04371	*OAS1*	5'-TTTCAGGATCAGTTAAATCGCC-3'	No. 51	5'-GGCCTGGCTGAATTACCCATG-3'	No. 52
127	U65785	*ORP150*	5'-GTTCTGCTCCTCCCAGACAG-3'	No. 53	5'-GCCCTAGCTCCTGCTACAGA-3'	No. 54
132	D38554	*PCOLN3*	5'-GCTCACTGCGTTTGGTTTTC-3'	No. 55	5'-CAGCATTCTAGGAGAAAGGTGAA-3'	No. 56
141	AA931981	*PPM1B*	5'-CTGTAACGTTTTCCTGAAGCTGT-3'	No. 57	5'-TCAGTACAGGGTTGGATCAGAGT-3'	No. 58
143	AF044588	*PRC1*	5'-GTGCCTACTTTGCCTGAGTTC-3'	No. 59	5'-CAGGACACGTACTGTATGAGGTAAA-3'	No. 60
149	AF043498	*PSCA*	5'-GACCATGTATGTTTGCACCC-3'	No. 61	5'-AACTCACGTCAACTCTTGTCCTC-3'	No. 62
152	M77836	*PYCR1*	5'-ATCCCAAGTCCAGCGTGAAG-3'	No. 63	5'-TCCACTATTCCACCCACAGTAAC-3'	No. 64
155	X64652	*RBMS1*	5'-CTGTCGAGACGTCTAATGACC-3'	No. 65	5'-TTACTAAAATAAACCTGTTCGGGGG-3'	No. 66
157	AA316525	*REGIV*	5'-CCAGTAGTGGCTTCTAGCTC-3'	No. 67	5'-GAAAAACAAGCAGGAGTTGAGTG-3'	No. 68
164	AA308062	*S100P*	5'-GCATGATCATAGACGTCTTTTCC-3'	No. 69	5'-GATGAACTCACTGAAGTCCACCT-3'	No. 70
169	AF029082	*SFN*	5'-GAGCGCACCTAACCACTGGTC-3'	No. 71	5'-TGAGTGTCACAGGGGAACTTTAT-3'	No. 72
170	AA639599	*SLC12A2*	5'-AACCGAAGTCTCCATACACG-3'	No. 73	5'-GTTCGTGGGAATCATCAGAG-3'	No. 74
173	K03195	*SLC2A1*	5'-AACCGAAGTCTCCATACACG-3'	No. 75	5'-GTTCGTGGGAATCATCAGAG-3'	No. 76
178	M32313	*SRD5A1*	5'-TCTGTAACAATAACAAGACC-3'	No. 77	5'-CCAGATGAGATGATAAGGCAAAG-3'	No. 78
180	M81601	*TCEA1*	5'-TGTCCCAAGTCTTATTTGCTGA-3'	No. 79	5'-GCAACAGTGGCCTTTAAAGTATG-3'	No. 80
184	K02581	*TK1*	5'-GTAATTGTGGCTGCACTGGAT-3'	No. 81	5'-ATTTCATAAGCTACAGCAGAGGC-3'	No. 82
188	U73379	*UBCH10*	5'-ACACACATGCTGCCGAGCTC-3'	No. 83	5'-TAATATACAAGGGCTCAACCGAG-3'	No. 84
196	AA581940	*WHSC1*	5'-CCTATGAGTGTAGTTGATGAC-3'	No. 85	5'-CAACTGGCAAGTCTCAACTCTCT-3'	No. 86
198	AA7	*FLJ1*	5'-	No.	5'-	No.

	09155	*0134*	TCCAGATGGATTTGTCCTGTATC-3'	87	TAGTAGCAAGCCCAGTAACCTTG-3'	88
199	AA806630	*FLJ10540*	5'-GCTTACCATTGAAACTTAACCCC-3'	No. 89	5'-CTCATTTACAGTAGCCCAGTGGT-3'	No. 90
203	AA918811	*FLJ20225*	5'-GACTTCCACAATGAACAGGGTAA-3'	No. 91	5'-ATTGGAATAAGAGGAACAGGAGC-3'	No. 92
208	D14657	*KIAA0101*	5'-CCAATTAGCTTTGTTGAACAGGC-3'	No. 93	5'-GGCAGCAGTACAACAATCTAAGC-3'	No. 94
217	R39794	*KIAA1624*	5'-CAGTGCTACACCCACTTCTTG-3'	No. 95	5'-ATACCACCAATGGTTCTGCTATG-3'	No. 96
218	AA434045	*KIAA1808*	5'-CTCATCTTTGAAGCCAGCAG-3'	No. 97	5'-GACTCACAGGCAGGAACATC-3'	No. 98
225	AA523117	*FLJ21504*	5'-GGATAGCTGGGGCATTTGTCTAG-3'	No. 99	5'-TCCATAAAAGAGTTTGGCAGTC-3'	No. 100
231	AA789332	*VANGL1*	5'-GAGTTGTATTATGAAGAGGCCGA-3'	No. 101	5'-ATGTCTCAGACTGTAAGCGAAGG-3'	No. 102
234	AI349804	*EST*	5'-GTAGATGTGGGGACAACAGAGAG-3'	No. 103	5'-TTTAAAGTCACCTTAGGTTGGGG-3'	No. 104
239	AA806114	*EST*	5'-CACCTATCCCTATTACCTGACCC-3'	No. 105	5'-TCTGAGGGTTTACATTGACGACT-3'	No. 106
242	AA419568	*EST*	5'-GAGTCCAGGTAAGTGAATCTGTCC-3'	No. 107	5'-ATTTCCACCGAGACCTCTCATC-3'	No. 108
245	AA570186	*EST*	5'-GTCTATCTGTGCTGGAACCTGAG-3'	No. 109	5'-GTGTAGGTGAGTGCTTTCTCCA-3'	No. 110
253	AA830326	*EST*	5'-ACTCCCGAGTAAATCATAGAGCC-3'	No. 111	5'-GACTGTTTCTACTCCAGAGGGGT-3'	No. 112
254	AI240520	*FXYD3*	5'-AAAGCTGATGAGGACAGACCAG-3'	No. 113	5'-GGCAGAGGCACAATCATTTTAG-3'	No. 114
259	AI027791	*EST*	5'-TGGTGTCTTTCTACCATTCAAGG-3'	No. 115	5'-AAAAGGCTAGTCCCCTTCTACCT-3'	No. 116
	AF141347	*TUBA*	5'-CTTGGGTCTGTAACAAAGCATTC-3'	No. 117	5'-AAGGATTATGAGGAGGTTGGTGT-3'	No. 118

Accession numbers and gene symbols were retrieved from the Unigene Databases (build#131).

EXAMPLE 2: IDENTIFICATION OF PNC – ASSOCIATED GENES

The up- or down-regulated genes were identified common to pancreatic cancer using following criteria; 1) genes which were able to obtain expression data in more than 50%

cancer cases, and 2) genes whose expression ratio was more than 5.0 in pancreatic cancer cells (defined as up-regulated genes) or genes whose expression ratio was under 0.2 (defined as down-regulated genes) in more than 50% of informative cases. Moreover, 3) the genes which were able to calculate in 33 to 50% cases and which expressed the expression ratio of more than 5.0 in all of that cases were also evaluated as up-regulated genes.

IDENTIFICATION OF GENES WITH CLINICALLY RELEVANT EXPRESSION PATTERNS IN PNC CELLS

The expression of approximately 23,000 genes in in 18 pancreatic cancer patients was examined using cDNA microarray. Individual data were excluded when both Cy5 and Cy3 signals were under cut-off values. Two hundred fifty-nine up-regulated genes were identified whose expression ratio was more than 5.0 in PNC cells (*see* Table 3). 167 of them were expressed greater than 10-fold comparing to the normal ductal cells. Three hundred forty-six down-regulated genes whose expression ratio was less than 0.2 were identified (*see* Table 4).

Among the up-regulated genes, interferon induced transmembrane protein 1 (IFITM1), plasminogen activator, urokinase (PLAU), prostate stem cell antigen (PSCA), S100 calcium binding protein P (S100P), RNA binding-motif single-stranded interacting protein 1 (RBMS1), and baculoviral IAP repeat-containing 5 (BIRC5), have been reported to be overexpressed in pancreatic cancer (5, 6). Furthermore, these up-regulated genes included ones encoding proteins involved in the signal transduction pathway, transcriptional factors, cell cycle, and cell adhesion (Table 5).

Significantly overexpressed genes have diagnostic potential, and of them which were critical for tumor growth have also therapeutic potential. Specifically, genes such as regenerating gene type IV (REGIV), ephrin type-A receptor 4 precursor (EphA4), and vang (van gogh, Drosophila)-like 1 (VANGL1), are useful as a potential molecular target for new therapeutic agents.

REGIV was over-expressed in all informative pancreatic cancer cases, and the overexpression was confirmed in 7 of the 12 pancreatic cancer cases by semi-quantitative RT-PCR. Since REGIV protein was thought to be a secreted protein from the amino-acid sequences and in fact its secretion was detected in the culture medium of HT29-5M12 cells (22), it is a candidate as tumor marker.

EphA4 was indicated to be overexpressed in 12 of the 14 informative pancreatic cancer cases in the microarray, and confirmed in 9 of the 12 cases were examined by semi-quantitative RT-PCR. EphA4 is known to be a membrane receptor belonging to the ephrin family, which contains an intracellular tyrosine kinase catalytic domain (23). Involvement of EphA4 in any human cancer has not been reported. However, its nature of the cytoplasmic membrane receptor protein with possible tyrosine kinase activity as well as high level expression in cancer cells suggest that EphA4 is a candidate gene for therapeutic agents.

VANGL1 was over-expressed if all of the informative pancreatic cancer cases in the microarray data, and its high expression was also confirmed in 9 of the 12 cases by semi-quantitative RT-PCR. VANGL1, which contained four putative transmembrane domains, was expressed specifically in testis and ovary among 29 normal tissues examined (4). This gene was also highly and frequency transactivated in hepatocellular carcinoma. Since the enforced reduction of this gene expression in hepatocellular carcinomas induced apoptosis (4), this gene product is a good candidate for development of novel anti-cancer drugs. Among the genes that were functionally highly overexpressed in pancreatic cancer such as the above mentioned genes, those whose products are putative membranous or secreted are of interest for potential as novel anti-cancer drugs or as serological diagnostic markers for early detection.

To confirm the reliability of the expression profiles indicated by microarray analysis, semi-quantitative RT-PCR experiments were performed. Other 55 genes whose cancer/normal ratios were highest among the informative genes, APP, ARHGDIB, ATDC, ATP1B3, BIRC5, BUB1B, CELSR3, CKS1, CKS2, CYP2S1, E2-EPF, ELF4, ENC1, Evi-1, FOXM1, GW112, GYS1, HDGF, HOXB7, hPAD-colony10, KNSL6, KPNB2, MMP11, MYBL2, OAS1, ORP150, PCOLN3, PPM1B, PRC1, PSCA, PYCR1, RBMS1, S100P, SFN, SLC12A2, SLC2A1, SRD5A1, TCEA1, TK1, UBCH10, WHSC1, FLJ10134, FLJ10540, FLJ20225, KIAA0101, KIAA1624, KIAA1808, FLJ21504, FXYD3, and 6 ESTs (Accession No.AI349804, AA806114, AA419568, AA570186, AA830326, AI027791) were PCR-amplified and compared with the microarray data. As shown in Fig.2, the results of the cDNA microarray werehighly similar to those of the RT-PCR analysis in the great majority of the tested cases.

APP was confirmed whose over-expression in 10 of the 12 cases,

ARHGDIB was confirmed whose over-expression in 12 cases,

ATDC was confirmed whose over-expression in 10 of the 12 cases,
ATP1B3 was confirmed whose over-expression in 12 cases,
BIRC5 was confirmed whose over-expression in 12 cases,
BUB1B was confirmed whose over-expression in 12 cases,
CELSR3 was confirmed whose over-expression in 9 of the 12 cases,
CKS1 was confirmed whose over-expression in 7 of the 12 cases,
CKS2 was confirmed whose over-expression in 11 of the 12 cases,
CYP2S1 was confirmed whose over-expression in 8 of the 12 cases,
E2-EPF was confirmed whose over-expression in 8 of the 12 cases,
ELF4 was confirmed whose over-expression in 11 of the 12 cases,
ENC1 was confirmed whose over-expression in 7 of the 12 cases,
Evi-1 was confirmed whose over-expression in 11 of the 12 cases,
FOXM1 was confirmed whose over-expression in 11 of the 12 cases,
GW112 was confirmed whose over-expression in 7 of the 12 cases,
GYS1 was confirmed whose over-expression in 10 of the 12 cases,
HDGF was confirmed whose over-expression in 10 of the 12 cases,
HOXB7 was confirmed whose over-expression in 6 of the 12 cases,
hPAD-colony10 was confirmed whose over-expression in 6 of the 12 cases,
KNSL6 was confirmed whose over-expression in 12 cases,
KPNB2 was confirmed whose over-expression in 10 of the 12 cases,
MMP11 was confirmed whose over-expression in 10 of the 12 cases,
MYBL2 was confirmed whose over-expression in 11 of the 12 cases,
OAS1 was confirmed whose over-expression in 10 of the 12 cases ,
ORP150 was confirmed whose over-expression in 8 of the 12 cases,
PCOLN3 was confirmed whose over-expression in 4 of the 12 cases,
PPM1B was confirmed whose over-expression in 3 of the 12 cases,
PRC1 was confirmed whose over-expression in 12 cases,
PSCA was confirmed whose over-expression in 6 of the 12 cases,
PYCR1 was confirmed whose over-expression in 9 of the 12 cases,
RBMS1 was confirmed whose over-expression in 12 cases,
S100P was confirmed whose over-expression in 10 of the 12 cases,
SFN was confirmed whose over-expression in 9 of the 12 cases,

SLC12A2 was confirmed whose over-expression in 5 of the 12 cases,
SLC2A1 was confirmed whose over-expression in 11 of the 12 cases,
SRD5A1 was confirmed whose over-expression in 8 of the 12 cases,
TCEA1 was confirmed whose over-expression in 8 of the 12 cases,
TK1 was confirmed whose over-expression in 10 of the 12 cases,
UBCH10 was confirmed whose over-expression in 10 of the 12 cases,
WHSC1 was confirmed whose over-expression in 8 of the 12 cases,
FLJ10134 was confirmed whose over-expression in 8 of the 12 cases,
FLJ10540 was confirmed whose over-expression in 11 of the 12 cases,
FLJ20225 was confirmed whose over-expression in 5 of the 12 cases,
KIAA0101 was confirmed whose over-expression in 12 cases,
KIAA1624 was confirmed whose over-expression in 9 of the 12 cases,
KIAA1808 was confirmed whose over-expression in 8 of the 12 cases,
FLJ21504 was confirmed whose over-expression in 11 of the 12 cases,
FXYD3 was confirmed whose over-expression in 9 of the 12 cases, and
Accession No.AI349804 was confirmed whose over-expression in 11 of the 12 cases,
AA806114 was confirmed whose over-expression in 8 of the 12 cases,
AA419568 was confirmed whose over-expression in 9 of the 12 cases,
AA570186 was confirmed whose over-expression in 6 of the 12 cases,
AA830326 was confirmed whose over-expression in 12 cases,
AI027791 was confirmed whose over-expression in 6 of the 12 cases.

These data verified the reliability of our strategy to identify commonly up-regulated genes in PNC cells.

Among the 346 down-regulated genes in pancreatic cancer cells, functions of 211 genes are characterized. These included genes that have been reported to be invoved in growth suppression (24,27,28,29), such as AXIN1 up-regulated 1(AXUD1), Deleted in liver cancer 1 (DLC1), growth arrest and DNA-damage-inducible, beta (GADD45B), and P53-inducible p53DINP1 (p53DINP1).

The down-regulated genes are likely to have a tumor suppressive function. Although the representative tumor suppressor genes for pancreatic cancer such as SMAD4, TP53, INK4A, and BRCA2 (24, 25) were not observed in down-regulated gene list, other genes that were reported to be involved in tumor suppression or apoptosis, such as, AXIN1 up-regulated

1(AXUD1), deleted in liver cancer 1 (DLC1), growth arrest and DNA-damage-inducible, beta (GADD45B), p53-inducible p53DINP1 (p53DINP1) were included in these data.

AXUD1, a nuclear protein, is induced in response to elevation of axin that is a key mediator of the Wnt-signalling pathway and is important in axis formation in early development. Dysfunction or down-regulation of the Wnt-signaling pathway is observed in human tumors, suggesting that this gene product has a tumor suppressor function (26, 27). Hence, these data imply that down-regulation of AXUD1 might lead to down-regulation of this signaling pathway and then lead to pancreatic carcinogenesis. Deleted in liver cancer 1 (DLC1) was suggested to be a candidate tumor suppressor gene for human liver cancer, as well as for prostate, lung, colorectal, and breast cancers. DLC1 shares high sequence similarity with the rat p122 RhoGap that negatively regulates the Rho GTPases. Hence, down-regulation of DLC1 is considered to result in the constitutive activation of the Rho-Rho-kinase pathway and subsequent oncogenic malignant transformation (28, 29).

Table3. A list of up-regulated genes

PNC Assignment	Accession No.	Symbol	Gene Name
1	V00478	*ACTB*	actin, beta
2	D26579	*ADAM8*	a disintegrin and metalloproteinase domain 8
3	D14874	*ADM*	adrenomedullin
4	H78430	*AHSG*	alpha-2-HS-glycoprotein
5	W92633	*AIB3*	thyroid hormone receptor binding protein
6	AF024714	*AIM2*	absent in melanoma 2
7	X60673	*AK3*	adenylate kinase 3
8	AF047002	*ALY*	transcriptional coactivator
9	AI341261	*ANLN*	anillin (Drosophila Scraps homolog), actin binding protein
10	J03578	*ANXA6*	annexin A6
11	U81504	*AP3B1*	adaptor-related protein complex 3, beta 1 subunit
12	AA916826	*APP*	amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer disease)
13	L20688	*ARHGDIB*	Rho GDP dissociation inhibitor (GDI) beta
14	AF006086	*ARPC3*	actin related protein 2/3 complex, subunit 3 (21 kD)
15	L24203	*ATDC*	ataxia-telangiectasia group D-associated protein
16	U51478	*ATP1B3*	ATPase, Na+/K+ transporting, beta 3 polypeptide
17	AA148566	*ATP2B4*	ATPase, Ca++ transporting, plasma membrane 4

18	W27948	*ATP6S1*	ATPase, H+ transporting, lysosomal (vacuolar proton pump), subunit 1
19	U75285	*BIRC5*	baculoviral IAP repeat-containing 5 (survivin)
20	L13689	*BMI1*	murine leukemia viral (bmi) oncogene homolog
21	W91908	*BRAG*	B cell RAG associated protein
22	AF068760	*BUB1B*	budding uninhibited by benzimidazoles 1 (yeast homolog), beta
23	AF028824	*C19ORF3*	chromosome 19 open reading frame 3
24	J04080	*C1S*	complement component 1, s subcomponent
25	M15082	*C2*	complement component 2
26	AA600048	*CALD1*	caldesmon 1
27	AA621719	*CAP-C*	chromosome-associated polypeptide C
28	AA557142	*CD2AP*	CD2-associated protein
29	Z11697	*CD83*	CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)
30	H52870	*CDC10*	CDC10 (cell division cycle 10, S. cerevisiae, homolog)
31	AA421724	*CDC20*	CDC20 (cell division cycle 20, S. cerevisiae, homolog)
32	X63629	*CDH3*	cadherin 3, type 1, P-cadherin (placental)
33	AB011536	*CELSR3*	cadherin, EGF LAG seven-pass G-type receptor 3, flamingo (Drosophila) homolog
34	X95404	*CFL1*	cofilin 1 (non-muscle)
35	X54941	*CKS1*	CDC28 protein kinase 1
36	X54942	*CKS2*	CDC28 protein kinase 2
37	AA001074	*CNNM4*	Cyclin M4
38	AA977821	*COL1A1*	collagen, type I, alpha 1
39	J03464	*COL1A2*	collagen, type I, alpha 2
40	X14420	*COL3A1*	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)
41	AI140851	*COL6A1*	collagen, type VI, alpha 1
42	J04823	*COX8*	cytochrome c oxidase subunit VIII
43	AA523543	*CRABP1*	cellular retinoic acid-binding protein 1
44	AA905901	*CRSP3*	cofactor required for Sp1 transcriptional activation, subunit 3 (130kD)
45	X16312	*CSNK2B*	casein kinase 2, beta polypeptide
46	U16306	*CSPG2*	chondroitin sulfate proteoglycan 2 (versican)
47	U40763	*CYP*	Clk-associating RS-cyclophilin
48	AA579959	*CYP2S1*	cytochrome P540 family member predicted from ESTs
49	AA863145	*DAO*	D-amino-acid oxidase
50	AI287670	*DDEF1*	Development and differentiation enhancing factor 1

51	AI159886	*DDX21*	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 21
52	U90426	*DDXL*	nuclear RNA helicase, DECD variant of DEAD box family
53	AA921756	*DIA4*	diaphorase (NADH/NADPH) (cytochrome b-5 reductase)
54	M91670	*E2-EPF*	ubiquitin carrier protein
55	AA457022	*E2IG5*	hypothetical protein, estradiol-induced
56	U32645	*ELF4*	E74-like factor 4 (ets domain transcription factor)
57	AF010314	*ENC1*	ectodermal-neural cortex (with BTB-like domain)
58	AF027299	*EPB41L2*	erythrocyte membrane protein band 4.1-like 2
59	L36645	*EPHA4*	EphA4
60	AA983304	*ERH*	enhancer of rudimentary (Drosophila) homolog
61	AI627919	*Evi-1*	ecotropic viral integration site 1
62	X02761	*FN1*	fibronectin 1
63	L16783	*FOXM1*	forkhead box M1
64	M14333	*FYN*	FYN oncogene related to SRC, FGR, YES
65	N36998	*GALNT2*	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2
66	AA418167	*GATA3*	GATA-binding protein 3
67	U78027	*GLA*	galactosidase, alpha
68	AF040260	*GMDS*	GDP-mannose 4,6-dehydratase
69	J03260	*GNAZ*	guanine nucleotide binding protein (G protein), alpha z polypeptide
70	D63997	*GOLGA3*	golgi autoantigen, golgin subfamily a, 3
71	X62320	*GRN*	granulin
72	D87119	*GS3955*	GS3955 protein
73	AA652197	*GW112*	differentially expressed in hematopoietic lineages
74	J04501	*GYS1*	glycogen synthase 1 (muscle)
75	M60756	*H2BFQ*	H2B histone family, member Q
76	AA608605	*HCS*	cytochrome c
77	D16431	*HDGF*	hepatoma-derived growth factor (high-mobility group protein 1-like)
78	X63187	*HE4*	epididymis-specific, whey-acidic protein type, four-disulfide core
79	AA714394	*HMG2*	high-mobility group (nonhistone chromosomal) protein 2
80	X92518	*HMGIC*	high-mobility group (nonhistone chromosomal) protein isoform I-C
81	X06985	*HMOX1*	heme oxygenase (decycling) 1
82	N92060	*HNRPL*	Heterogeneous nuclear ribonucleoprotein L
83	M16937	*HOXB7*	homeo box B7
84	AA495868	*hPAD-colony10*	peptidylarginine deiminase type I

85	AF070616	*HPCAL1*	hippocalcin-like 1
86	AF064084	*ICMT*	isoprenylcysteine carboxyl methyltransferase
87	AA328385	*ICSBP1*	interferon consensus sequence binding protein 1
88	AA573936	*IDH2*	isocitrate dehydrogenase 2 (NADP+), mitochondrial
89	AI341760	*IFI27*	interferon, alpha-inducible protein 27
90	AI081175	*IFITM1*	interferon induced transmembrane protein 1 (9-27)
91	X16302	*IGFBP2*	insulin-like growth factor binding protein 2 (36kD)
92	M87789	*IGHG3*	immunoglobulin heavy constant gamma 3 (G3m marker)
93	M87790	*Igl*λ	immunoglobulin lambda locus
94	S74221	*IK*	IK cytokine, down-regulator of HLA II
95	X59770	*IL1R2*	interleukin 1 receptor, type II
96	J05272	*IMPDH1*	IMP (inosine monophosphate) dehydrogenase 1
97	AB003184	*ISLR*	immunoglobulin superfamily containing leucine-rich repeat
98	M15395	*ITGB2*	integrin, beta 2
99	L38961	*ITM1*	integral membrane protein 1
100	AA574178	*KAI1*	Kangai 1
101	M55513	*KCNA5*	potassium voltage-gated channel, shaker-related subfamily, member 5
102	U63743	*KNSL6*	kinesin-like 6 (mitotic centromere-associated kinesin)
103	U70322	*KPNB2*	karyopherin (importin) beta 2
104	J00269	*KRT6A*	keratin 6A
105	X53305	*LAP18*	leukemia-associated phosphoprotein p18 (stathmin)
106	AA742701	*LCP1*	lymphocyte cytosolic protein 1 (L-plastin)
107	AA826336	*LHFPL2*	lipoma HMGIC fusion partner-like 2
108	U24576	*LMO4*	LIM domain only 4
109	AA555023	*LOC51191*	cyclin-E binding protein 1
110	AI299952	*LOC51765*	serine/threonine protein kinase MASK
111	U89942	*LOXL2*	lysyl oxidase-like 2
112	U15128	*MGAT2*	mannosyl (alpha,6-)-glycoprotein beta,2-N-acetylglucosaminyltransferase
113	J03746	*MGST1*	microsomal glutathione S-transferase 1
114	AA531437	*MLLT4*	myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 4
115	X57766	*MMP11*	matrix metalloproteinase 11 (stromelysin 3)
116	J05070	*MMP9*	matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase)
117	AF034374	*MOCS1*	molybdenum cofactor biosynthesis protein A; molybdenum cofactor biosynthesis protein C

118	M74905	*MPG*	N-methylpurine-DNA glycosylase
119	AA458825	*MTIF2*	mitochondrial translational initiation factor 2
120	X13293	*MYBL2*	v-myb avian myeloblastosis viral oncogene homolog-like 2
121	D32002	*NCBP1*	nuclear cap binding protein subunit 1, 80kD
122	AA729022	*NCOA3*	nuclear receptor coactivator 3
123	AF047434	*NDUFS5*	NADH dehydrogenase (ubiquinone) Fe-S protein 5 (15kD) (NADH-coenzyme Q reductase)
124	AA602490	*NOP5/NOP58*	nucleolar protein NOP5/NOP58
25	X04371	*OAS1*	2',5'-oligoadenylate synthetase 1 (40-46 kD)
126	M23204	*OAT*	ornithine aminotransferase (gyrate atrophy)
127	U65785	*ORP150*	oxygen regulated protein (150kD)
128	AI223298	*P125*	Sec23-interacting protein p125
129	M24486	*P4HA1*	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I
130	M80482	*PACE4*	paired basic amino acid cleaving system 4
131	L11370	*PCDH1*	protocadherin 1 (cadherin-like 1)
132	D38554	*PCOLN3*	procollagen (type III) N-endopeptidase
133	AA034069	*PDK1*	pyruvate dehydrogenase kinase, isoenzyme 1
134	AA586974	*PI3*	protease inhibitor 3, skin-derived (SKALP)
135	M16750	*PIM1*	pim oncogene
136	AA234962	*PKP3*	plakophilin 3
137	X02419	*PLAU*	plasminogen activator, urokinase
138	AA308562	*PLEK2*	pleckstrin 2 (mouse) homolog
139	U97519	*PODXL*	podocalyxin-like
140	AI185998	*PPIC*	peptidylprolyl isomerase C (cyclophilin C)
141	AA931981	*PPM1B*	protein phosphatase 1B (formerly 2C), magnesium-dependent, beta isoform
142	L42373	*PPP2R5A*	protein phosphatase 2, regulatory subunit B (B56), alpha isoform
143	AF044588	*PRC1*	protein regulator of cytokinesis 1
144	X74496	*PREP*	prolyl endopeptidase
145	M65066	*PRKAR1B*	protein kinase, cAMP-dependent, regulatory, type I, beta
146	AA972414	*PRO2975*	hypothetical protein PRO2975
147	D00860	*PRPS1*	phosphoribosyl pyrophosphate synthetase 1
148	D87258	*PRSS11*	protease, serine, 11 (IGF binding)
149	AF043498	*PSCA*	prostate stem cell antigen
150	D26598	*PSMB3*	proteasome (prosome, macropain) subunit, beta type, 3
151	X62006	*PTB*	polypyrimidine tract binding protein (heterogeneous nuclear ribonucleoprotein I)

152	M77836	*PYCR1*	pyrroline-5-carboxylate reductase 1
153	X12953	*RAB2*	RAB2, member RAS oncogene family
154	AA346311	*RAI3*	retinoic acid induced 3
155	X64652	*RBMS1*	RNA binding motif, single stranded interacting protein 1
156	S45545	*RCV1*	recoverin
157	AA316525	*REGIV*	Regenerating gene type IV
158	AB008109	*RGS5*	regulator of G-protein signalling 5
159	AA778308	*RNASE1*	ribonuclease, RNase A family, 1 (pancreatic)
160	AA811043	*RNASE6PL*	ribonuclease 6 precursor
161	L05096	*RPL39*	Homo sapiens ribosomal protein L39 mRNA, complete cds
162	X76302	*RY1*	putative nucleic acid binding protein RY
163	D38583	*S100A11*	S100 calcium-binding protein A11 (calgizzarin)
164	AA308062	*S100P*	S100 calcium-binding protein P
165	AA452018	*SCD*	stearoyl-CoA desaturase (delta-9-desaturase)
166	D49737	*SDHC*	succinate dehydrogenase complex, subunit C, integral membrane protein, 15kD
167	AA579861	*SEC23A*	Sec23 (S. cerevisiae) homolog A
168	AA430643	*SEPW1*	selenoprotein W, 1
169	AF029082	*SFN*	stratifin
170	AA639599	*SLC12A2*	solute carrier family 12 (sodium/potassium/chloride transporters), member 2
171	L20859	*SLC20A1*	solute carrier family 20 (phosphate transporter), member 1
172	L02785	*SLC26A3*	solute carrier family 26, member 3
173	K03195	*SLC2A1*	solute carrier family 2 (facilitated glucose transporter), member 1
174	U09873	*SNL*	singed (Drosophila)-like (sea urchin fascin homolog like)
175	X13482	*SNRPA1*	small nuclear ribonucleoprotein polypeptide A'
176	M37716	*SNRPE*	small nuclear ribonucleoprotein polypeptide E
177	J03040	*SPARC*	secreted protein, acidic, cysteine-rich (osteonectin)
178	M32313	*SRD5A1*	steroid-5-alpha-reductase, alpha polypeptide 1
179	M95787	*TAGLN*	transgelin
180	M81601	*TCEA1*	transcription elongation factor A (SII), 1
181	AF033095	*TEGT*	testis enhanced gene transcript (BAX inhibitor 1)
182	L12350	*THBS2*	thrombospondin 2
183	M77142	*TIA1*	TIA1 cytotoxic granule-associated RNA-binding protein
184	K02581	*TK1*	thymidine kinase 1, soluble
185	AA429631	*TK2*	thymidine kinase 2, mitochondrial
186	U09087	*TMPO*	thymopoietin

187	AF065388	*TSPAN*	tetraspan 1
188	U73379	*UBCH10*	ubiquitin carrier protein E2-C
189	AA977545	*UBE2D2*	ubiquitin-conjugating enzyme E2D 2 (homologous to yeast UBC4/5)
190	U45328	*UBE2I*	ubiquitin-conjugating enzyme E2I (homologous to yeast UBC9)
191	M57899	*UGT1A1*	UDP glycosyltransferase 1 family, polypeptide A1
192	AA315189	*UQCRB*	ubiquinol-cytochrome c reductase binding protein
193	AB000450	*VRK2*	vaccinia related kinase 2
194	AA079060	*WFDC2*	WAP four-disulfide core domain 2
195	AA043277	*WFS1*	Wolfram syndrome 1 (wolframin)
196	AA581940	*WHSC1*	Wolf-Hirschhorn syndrome candidate 1
197	AI185056	*ZNF134*	zinc finger protein 134 (clone pHZ5)
198	AA709155	*FLJ10134*	hypothetical protein FLJ10134
199	AA806630	*FLJ10540*	hypothetical protein FLJ10540
200	AA115015	*FLJ10633*	hypothetical protein FLJ10633
201	AA394229	*FLJ10637*	hypothetical protein FLJ10637
202	AA633302	*FLJ20063*	hypothetical protein FLJ20063
203	AA918811	*FLJ20225*	hypothetical protein
204	R09189	*FLJ20281*	hypothetical protein FLJ20281
205	AA112198	*FLJ20296*	hypothetical protein FLJ20296
206	AI033837	*FLJ20406*	hypothetical protein FLJ20406
207	AA974462	*FLJ23053*	hypothetical protein FLJ23053
208	D14657	*KIAA0101*	KIAA0101 gene product
209	D61862	*KIAA0332*	KIAA0332 protein
210	AB014566	*KIAA0666*	KIAA0666 protein
211	AB014570	*KIAA0670*	KIAA0670 protein/acinus
212	AA665890	*KIAA0729*	KIAA0729 protein
213	W80765	*KIAA0731*	KIAA0731 protein
214	AF052170	*KIAA0750*	KIAA0750 gene product
215	D20853	*KIAA0776*	KIAA0776 protein
216	AA031775	*KIAA0990*	KIAA0990 protein
217	R39794	*KIAA1624*	KIAA1624 protein
218	AA434045	*KIAA1808*	ESTs
219	AI074410	*KIAA1863*	Homo sapiens cDNA FLJ13996 fis, clone Y79AA1002211
220	AF070638	*CGI-57*	hypothetical protein
221	N38882		H.sapiens gene from PAC 106H8
222	AI142828		Homo sapiens adlican mRNA, complete cds

223	AA028961		Homo sapiens cDNA FLJ12150 fis, clone MAMMA1000422
224	AA933635		Homo sapiens cDNA FLJ13154 fis, clone NT2RP3003427
225	AA523117	*FLJ21504*	Homo sapiens cDNA: FLJ21504 fis, clone COL05662
226	AA555187		Homo sapiens cDNA: FLJ22277 fis, clone HRC03740
227	AF035315		Homo sapiens clone 23664 and 23905 mRNA sequence
228	AA968840		Homo sapiens HSPC285 mRNA, partial cds
229	R55322		Homo sapiens mRNA; cDNA DKFZp547K204 (from clone DKFZp547K204)
230	W55876		Homo sapiens mRNA; cDNA DKFZp586A0424 (from clone DKFZp586A0424)
231	AA789332	*VANGL1*	ESTs, Moderately similar to KIAA1215 protein [H.sapiens]
232	AI310156		ESTs, Weakly similar to A4P_HUMAN INTESTINAL MEMBRANE A4 PROTEIN [H.sapiens]
233	C01335		ESTs, Weakly similar to FLDED [H.sapiens]
234	AI349804		ESTs, Weakly similar to IQGA_HUMAN RAS GTPASE-ACTIVATING-LIKE PROTEIN IQGAP1
235	AA683373		ESTs
236	H28960		ESTs
237	AA429665		ESTs
238	R17093		ESTs
239	AA806114		ESTs
240	AA707966		ESTs
241	D85376		ESTs
242	AA419568		ESTs
243	AA251355		ESTs
244	W63676		ESTs
245	AA570186		ESTs
246	AI239432		ESTs
247	AI264318		ESTs
248	AA553741		ESTs
249	N70804		ESTs
250	R61891		ESTs
251	W01507		ESTs
252	AA587884		ESTs
253	AA830326		ESTs
254	AI240520		ESTs
255	AA453716		ESTs
256	AI199761		ESTs

257	AI271678	ESTs
258	AA242941	ESTs
259	AI027791	ESTs

Table4 A list of down-regulated genes

PNC Assignment	Accession No.	Symbol	Gene Name
260	D16294	*ACAA2*	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)
261	M12963	*ADH1*	alcohol dehydrogenase 1 (class I), alpha polypeptide
262	X04299	*ADH3*	alcohol dehydrogenase 3 (class I), gamma polypeptide
263	L22214	*ADORA1*	adenosine A1 receptor
264	U04241	*AES*	amino-terminal enhancer of split
265	AF044961	*AKR1B11*	aldo-keto reductase family 1, member B11
266	U05861	*AKR1C1*	aldo-keto reductase family 1, member C1
267	D26125	*AKR1C4*	aldo-keto reductase family 1, member C4
268	AI765873	*ALDH10*	aldehyde dehydrogenase 10 (fatty aldehyde dehydrogenase)
269	X02747	*ALDOB*	aldolase B, fructose-bisphosphate
270	M18786	*AMY1A*	amylase, alpha 1A; salivary
271	M28443	*AMY2A*	amylase, alpha 2A; pancreatic
272	M22324	*ANPEP*	alanyl (membrane) aminopeptidase
273	Z11502	*ANXA13*	annexin A13
274	M82809	*ANXA4*	annexin A4
275	D00097	*APCS*	amyloid P component, serum
276	M30704	*AREG*	amphiregulin (schwannoma-derived growth factor)
277	AB007884	*ARHGEF9*	Cdc42 guanine exchange factor (GEF) 9
278	AI147612	*ARL7*	ADP-ribosylation factor-like 7
279	X83573	*ARSE*	arylsulfatase E (chondrodysplasia punctata 1)
280	L19871	*ATF3*	activating transcription factor 3
281	Y15724	*ATP2A3*	ATPase, Ca++ transporting, ubiquitous
282	AI091372	*AXUD1*	AXIN1 up-regulated
283	X83107	*BMX*	BMX non-receptor tyrosine kinase
284	AA468538	*BRPF3*	bromodomain and PHD finger containing, 3
285	U03274	*BTD*	biotinidase
286	D31716	*BTEB1*	basic transcription element binding protein 1
287	W45244	*C3*	complement component 3
288	J03037	*CA2*	carbonic anhydrase II

289	U36448	*CADPS*	Ca2+-dependent activator protein for secretion
290	AI085802	*CAV2*	Caveolin 2
291	J02988	*CD28*	CD28 antigen (Tp44)
292	M55509	*CES1*	carboxylesterase 1 (monocyte/macrophage serine esterase 1)
293	U91543	*CHD3*	chromodomain helicase DNA binding protein 3
294	AA417345	*CHP1*	chord domain-containing protein 1
295	U62431	*CHRNA2*	cholinergic receptor, nicotinic, alpha polypeptide 2 (neuronal)
296	U89916	*CLDN10*	claudin 10
297	AA885961	*CLDN2*	Claudin 2
298	J02883	*CLPS*	colipase, pancreatic
299	M64722	*CLU*	clusterin
300	X67318	*CPA1*	carboxypeptidase A1 (pancreatic)
301	U19977	*CPA2*	carboxypeptidase A2 (pancreatic)
302	AA780301	*CTSF*	cathepsin F
303	T84490	*CUGBP2*	CUG triplet repeat, RNA-binding protein 2
304	M22865	*CYB5*	cytochrome b-5
305	Y00498	*CYP2C8*	cytochrome P450, subfamily IIC (mephenytoin 4-hydroxylase), polypeptide 8
306	J04813	*CYP3A5*	cytochrome P450, subfamily IIIA (niphedipine oxidase), polypeptide 5
307	D00408	*CYP3A7*	cytochrome P450, subfamily IIIA, polypeptide 7
308	AA316159	*DC11*	DC11 protein
309	AA640753	*DDAH1*	dimethylarginine dimethylaminohydrolase 1
310	X96484	*DGCR6*	DiGeorge syndrome critical region gene 6
311	W76197	*DLC1*	Deleted in liver cancer 1
312	X68277	*DUSP1*	dual specificity phosphatase 1
313	M62829	*EGR1*	early growth response 1
314	M16652	*ELA1*	elastase 1, pancreatic
315	AA845162	*ELA3*	elastase 3, pancreatic (protease E)
316	M81635	*EPB72*	erythrocyte membrane protein band 7.2 (stomatin)
317	M16967	*F5*	coagulation factor V (proaccelerin, labile factor)
318	AA573905	*FCGBP*	Fc fragment of IgG binding protein
319	AA033657	*FGFR2*	fibroblast growth factor receptor 2
320	U20391	*FOLR1*	folate receptor 1 (adult)
321	U50743	*FXYD2*	FXYD domain-containing ion transport regulator 2
322	M11321	*GC*	mRNA for group supecific component (GC)
323	Y15409	*G6PT1*	glucose-6-phosphatase, transport (glucose-6-phosphate) protein 1

324	AA279817	*GADD45B*	growth arrest and DNA-damage-inducible, beta
325	L13720	*GAS6*	growth arrest-specific 6
326	S68805	*GATM*	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
327	M24903	*GGT1*	gamma-glutamyltransferase 1
328	AW008481	*GLUD1*	glutamate dehydrogenase 1
329	T79836	*GPS2*	G protein pathway suppressor 2
330	D86962	*GRB10*	growth factor receptor-bound protein 10
331	L76687	*GRB14*	growth factor receptor-bound protein 14
332	D49742	*HABP2*	hyaluronan-binding protein 2
333	W37916	*HCF-2*	host cell factor 2
334	U63008	*HGD*	homogentisate 1,2-dioxygenase (homogentisate oxidase)
335	W95267	*HIBADH*	3-hydroxyisobutyrate dehydrogenase
336	K01505	*HLA-DQA1*	DC classII histocompatibility antigen alpha-chain
337	M81141	*HLA-DQB1*	major histocompatibility complex, class II, DQ beta 1
338	J03048	*HPX*	hemopexin
339	T55714	*HS3ST1*	heparan sulfate (glucosamine) 3-O-sulfotransferase 1
340	AA206625	*HS6ST*	heparan sulfate 6-O-sulfotransferase
341	U14631	*HSD11B2*	hydroxysteroid (11-beta) dehydrogenase 2
342	M11717	*HSPA1A*	heat shock 70kD protein 1A
343	D49547	*HSPF1*	heat shock 40kD protein 1
344	AA885758	*HTATIP*	HIV Tat interactive protein, 60 kDa
345	M27492	*IL1R1*	interleukin 1 receptor, type I
346	AF014398	*IMPA2*	inositol(myo)(or 4)-monophosphatase 2
347	U84400	*INPP5D*	inositol polyphosphate-5-phosphatase, 145kD
348	AA345854	*ITGA3*	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
349	AA845511	*KCNJ16*	potassium inwardly-rectifying channel, subfamily J, member 16
350	AI025297	*KLF7*	Kruppel-like factor 7 (ubiquitous)
351	X79683	*LAMB2*	laminin, beta 2 (laminin S)
352	X77196	*LAMP2*	lysosomal-associated membrane protein 2
353	M87842	*LGALS2*	lectin, galactoside-binding, soluble, 2 (galectin 2)
354	AI160184	*LOC51673*	brain specific protein
355	AI093595	*LOC55895*	22kDa peroxisomal membrane protein-like
356	AA347844	*LOC56908*	Meis (mouse) homolog 2
357	AK025620	*LOC56990*	non-kinase Cdc42 effector protein SPEC2
358	AA461526	*LRRFIP2*	leucine rich repeat (in FLII) interacting protein 2
359	H17536	*LSM4*	U6 snRNA-associated Sm-like protein

360	AI092885	*LSM6*	Sm protein F
361	AA157731	*MAP1ALC3*	Microtubule-associated proteins 1A and 1B, light chain 3
362	X69078	*MAT1A*	methionine adenosyltransferase 1 alpha
363	X63380	*MEF2B*	MADS box transcription enhancer factor 2, polypeptide B (myocyte enhancer factor 2B)
364	L08895	*MEF2C*	MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)
365	X56741	*MEL*	mel transforming oncogene (derived from cell line NK14)-RAB8 homolog
366	AI037890	*MMP1*	matrix metalloproteinase 1 (interstitial collagenase)
367	R59292	*MS4A8B*	Membrane-spanning 4-domains, subfamily A, member 8B
368	AL022315	*MSE55*	serum constituent protein
369	D49441	*MSLN*	mesothelin
370	M74178	*MST1*	macrophage stimulating 1
371	U35113	*MTA1*	metastasis associated 1
372	Y09788	*MUC5B*	mucin 5, subtype B, tracheobronchial
373	AI745345	*MVP*	major vault protein
374	X69090	*MYOM1*	myomesin 1 (skelemin) (185kD)
375	AA497062	*NFIC*	nuclear factor I/C (CCAAT-binding transcription factor)
376	AI309212	*NLGN1*	neuroligin 1
377	AJ005282	*NPR2*	natriuretic peptide receptor B/guanylate cyclase B (atrionatriuretic peptide receptor B)
378	AA340728	*NR2F2*	nuclear receptor subfamily 2, group F, member 2
379	L13740	*NR4A1*	nuclear receptor subfamily 4, group A, member 1
380	X75918	*NR4A2*	nuclear receptor subfamily 4, group A, member 2
381	AB002341	*NRCAM*	neuronal cell adhesion molecule
382	AA435678	*P28*	dynein, axonemal, light intermediate polypeptide
383	AA576089	*p53DINP1*	P53-inducible p53DINP1
384	L15533	*PAP*	pancreatitis-associated protein
385	T56982	*PDE7A*	phosphodiesterase 7A
386	C05229	*PDK4*	pyruvate dehydrogenase kinase, isoenzyme 4
387	N47861	*PDP*	pyruvate dehydrogenase phosphatase
388	AF012281	*PDZK1*	PDZ domain containing 1
389	AA220941	*PHB*	prohibitin
390	D38616	*PHKA2*	phosphorylase kinase, alpha 2 (liver)
391	L47738	*PIR121*	p53 inducible protein
392	X98654	*PITPNM*	phosphatidylinositol transfer protein, membrane-associated
393	W19216	*PKIG*	protein kinase (cAMP-dependent, catalytic) inhibitor gamma

394	AF064594	*PLA2G6*	phospholipase A2, group VI (cytosolic, calcium-independent)
395	AF038440	*PLD2*	phospholipase D2
396	D87810	*PMM1*	phosphomannomutase 1
397	J05125	*PNLIP*	pancreatic lipase
398	Z11898	*POU5F1*	POU domain, class 5, transcription factor 1
399	AI343963	*PP2135*	PP2135 protein
400	U57961	*13CDNA73*	putative gene product
401	AI094447	*PP5395*	hypothetical protein PP5395
402	S74349	*PPARA*	peroxisome proliferative activated receptor, alpha
403	AB007851	*PRPSAP2*	phosphoribosyl pyrophosphate synthetase-associated protein 2
404	AA845165	*PRSS1*	protease, serine, 1 (trypsin 1)
405	D88378	*PSMF1*	proteasome (prosome, macropain) inhibitor subunit 1 (PI31)
406	U68142	*RAB2L*	RAB2, member RAS oncogene family-like
407	AI277086	*RAGB*	GTP-binding protein ragB
408	AA972852	*RBP1*	retinol-binding protein 1, cellular
409	X00129	*RBP4*	retinol-binding protein 4, interstitial
410	AA807607	*RDGBB*	retinal degeneration B beta
411	AA428540	*REC8*	Rec8p
412	M18963	*REG1A*	regenerating islet-derived 1 alpha (pancreatic stone protein, pancreatic thread protein)
413	AC004003	*RIPK2*	receptor-interacting serine-threonine kinase 2
414	AI341482	*RNB6*	RNB6
415	AW510670	*RNF3*	ring finger protein 3
416	U38894	*ROR1*	receptor tyrosine kinase-like orphan receptor 1
417	X65463	*RXRB*	retinoid X receptor, beta
418	U72355	*SAFB*	scaffold attachment factor B
419	AI338007	*SCDGF-B*	Spinal cord-derived growth factor-B
420	AA911283	*SCMH1*	sex comb on midleg homolog 1
421	U84487	*SCYD1*	small inducible cytokine subfamily D (Cys-X3-Cys), member 1 (fractalkine, neurotactin)
422	W73992	*SDCCAG43*	serologically defined colon cancer antigen 43
423	U28369	*SEMA3B*	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B
424	U38276	*SEMA3F*	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F
425	AI026695	*SENP1*	Sentrin/SUMO-specific protease
426	Z11793	*SEPP1*	selenoprotein P, plasma, 1

427	H89783	*SERPINA4*	serine (or cysteine) proteinase inhibitor, clade A (alpha antiproteinase, antitrypsin), member 4
428	J02943	*SERPINA6*	serine (or cysteine) proteinase inhibitor, clade A (alpha antiproteinase, antitrypsin), member 6
429	M13690	*SERPING1*	serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1
430	AF017988	*SFRP5*	secreted frizzled-related protein 5
431	N56912	*SFTPC*	surfactant, pulmonary-associated protein C
432	Y10032	*SGK*	serum/glucocorticoid regulated kinase
433	AI198522	*SLC11A3*	solute carrier family 11, member 3
434	U59299	*SLC16A5*	solute carrier family 16, member 5
435	AA243675	*SLC1A1*	solute carrier family 1, member 1
436	AA435777	*SLC25A1*	Solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1
437	NM_000340	*SLC2A2*	solute carrier family 2 (facilitated glucose transporter), member 2
438	M95548	*SLC3A1*	solute carrier family 3 (cystine, dibasic and neutral amino acid transporters, activator of cystine, dibasic and neutral amino acid transport), member 1
439	AF007216	*SLC4A4*	solute carrier family 4, sodium bicarbonate cotransporter, member 4
440	M24847	*SLC5A1*	solute carrier family 5 (sodium/glucose cotransporter), member 1
441	AA902273	*SMARCD3*	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, member 3
442	U41303	*SNRPN*	small nuclear ribonucleoprotein polypeptide N
443	AA604446	*SPINK5*	serine protease inhibitor, Kazal type, 5
444	J04765	*SPP1*	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)
445	L14865	*SSTR5*	somatostatin receptor 5
446	R60028	*TAB1*	transforming growth factor beta-activated kinase-binding protein 1
447	X58840	*TCF2*	transcription factor 2, hepatic; LF-B3; variant hepatic nuclear factor
448	J05068	*TCN1*	transcobalamin I (vitamin B12 binding protein, R binder family)
449	L15203	*TFF3*	trefoil factor 3 (intestinal)
450	D29992	*TFPI2*	tissue factor pathway inhibitor 2
451	AA403273	*TLE1*	transducin-like enhancer of split 1, homolog of Drosophila E(sp1)
452	U31449	*TM4SF4*	transmembrane 4 superfamily member 4
453	AA131918	*TMEM3*	transmembrane protein 3
454	U70321	*TNFRSF14*	tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator)
455	L21715	*TNNI2*	troponin I, skeletal, fast

456	AI091425	*TONDU*	TONDU
457	U54831	*TOP2B*	topoisomerase (DNA) II beta (180kD)
458	U44427	*TPD52L1*	tumor protein D52-like 1
459	M10605	*TTR*	transthyretin (prealbumin, amyloidosis type I)
460	AI090567	*TUBB2*	tubulin, beta, 2
461	L13852	*UBE1L*	ubiquitin-activating enzyme E1-like
462	X63359	*UGT2B10*	UDP glycosyltransferase 2 family, polypeptide B10
463	J05428	*UGT2B7*	UDP glycosyltransferase 2 family, polypeptide B7
464	AA446913	*USP11*	ubiquitin specific protease 11
465	L13288	*VIPR1*	vasoactive intestinal peptide receptor 1
466	D78298	*VLCAD*	very-long-chain acyl-CoA dehydrogenase
467	AA769424	*VNN2*	vanin 2
468	AF039022	*XPOT*	exportin, tRNA (nuclear export receptor for tRNAs)
469	D83407	*ZAKI4*	Down syndrome critical region gene 1-like 1
470	Z19002	*ZNF145*	zinc finger protein 145 (Kruppel-like, expressed in promyelocytic leukemia)
471	M58297	*ZNF42*	zinc finger protein 42 (myeloid-specific retinoic acid-responsive)
472	N24911	*C11ORF2*	chromosome 11 open reading frame2
473	AI186263	*C21ORF11*	chromosome 21 open reading frame 11
474	Y11392	*C21ORF2*	chromosome 21 open reading frame 2
475	H16793	*C8ORF4*	chromosome 8 open reading frame 4
476	AI160590	*DKFZp434G0522*	hypothetical protein DKFZp434G0522
477	T65389	*DKFZP434J214*	DKFZP434J214 protein
478	H61870	*DKFZP564F1123*	DKFZP564F1123 protein
479	AI218000	*DKFZP564K1964*	DKFZP564K1964 protein
480	AI306435	*DKFZP586A0522*	DKFZP586A0522 protein
481	W05570	*DKFZP586B0621*	DKFZP586B0621 protein
482	N92489	*FLJ10103*	hypothetical protein FLJ10103
483	AA933772	*FLJ10252*	hypothetical protein FLJ10252
484	AA452368	*FLJ10582*	hypothetical protein FLJ10582
485	AA481246	*FLJ12287*	hypothetical protein FLJ12287 similar to semaphorins
486	AI042204	*FLJ12895*	hypothetical protein FLJ12895
487	AI342612	*FLJ20011*	hypothetical protein FLJ20011
488	AA708532	*FLJ20041*	hypothetical protein FLJ20041

489	AI016890	*FLJ20542*	hypothetical protein FLJ20542
490	AA593701	*FLJ21817*	hypothetical protein FLJ21817 similar to Rhoip2
491	NM_022493	*FLJ21988*	hypothetical protein FLJ21988
492	N30915	*FLJ22649*	hypothetical protein FLJ22649 similar to signal peptidase SPC22/23
493	AA650281	*FLJ23153*	Likely ortholog of mouse tumor necrosis-alpha-induced adipose-related protein
494	AA522448	*FLJ23239*	hypothetical protein FLJ23239
495	AA403120	*HT014*	HT014
496	D31884	*KIAA0063*	KIAA0063 gene product
497	D87465	*KIAA0275*	KIAA0275 gene product
498	AI190847	*KIAA0397*	KIAA0397 gene product
499	AB011115	*KIAA0543*	KIAA0543 protein
500	AA910738	*KIAA0579*	KIAA0579 protein
501	AA156717	*KIAA0668*	KIAA0668 protein
502	W56303	*KIAA0802*	KIAA0802 protein
503	AA127777	*KIAA1071*	KIAA1071 protein
504	AI148832	*KIAA1209*	KIAA1209 protein
505	AA573892	*KIAA1359*	KIAA1359 protein
506	N54300	*KIAA1500*	KIAA1500 protein
507	N36929	*KIAA1954*	KIAA1954 protein
508	AA477232	*LOC56997*	hypothetical protein, clone Telethon(Italy_B41)_Strait02270_FL142
509	AF001550	*LOC57146*	hypothetical protein from clone 24796
510	AA303231	*LOC64744*	hypothetical protein AL133206
511	AA044186		Homo sapiens cDNA FLJ11410 fis, clone HEMBA1000852
512	D62873		Homo sapiens cDNA FLJ12900 fis, clone NT2RP2004321
513	AA858162		Homo sapiens cDNA FLJ13005 fis, clone NT2RP3000441
514	AA327291		Homo sapiens cDNA FLJ13322 fis, clone OVARC1001713
515	AI096874		Homo sapiens cDNA FLJ14115 fis, clone MAMMA1001760
516	H28758		Homo sapiens cDNA: FLJ20925 fis, clone ADSE00963
517	T04932		Homo sapiens cDNA: FLJ21545 fis, clone COL06195
518	AK025906		Homo sapiens cDNA: FLJ22253 fis, clone HRC02763
519	AI344138		Homo sapiens cDNA: FLJ22288 fis, clone HRC04157
520	AA206578		Homo sapiens cDNA: FLJ22316 fis, clone HRC05262
521	R89624		Homo sapiens cDNA: FLJ22386 fis, clone HRC07619
522	AA404225		Homo sapiens cDNA: FLJ22418 fis, clone HRC08590
523	AI089485		Homo sapiens cDNA: FLJ22479 fis, clone HRC10831

524	AA505312		Homo sapiens cDNA: FLJ22648 fis, clone HSI07329
525	R72460		Homo sapiens cDNA: FLJ22807 fis, clone KAIA2887
526	AA019961		Homo sapiens cDNA: FLJ22811 fis, clone KAIA2944
527	N46856		Homo sapiens cDNA: FLJ23091 fis, clone LNG07220
528	AA321321		Homo sapiens cDNA: FLJ23091 fis, clone LNG07220
529	AI084531		Homo sapiens cDNA: FLJ23093 fis, clone LNG07264
530	AA543086		Homo sapiens cDNA: FLJ23270 fis, clone COL10309
531	AA741042		Homo sapiens cDNA: FLJ23527 fis, clone LNG05966
532	AF009314		Homo sapiens clone TUA8 Cri-du-chat region mRNA
533	AA293837		Homo sapiens GKAP42 (FKSG21) mRNA, complete cds
534	AA195740		Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 41832
535	AA829835		Homo sapiens mRNA; cDNA DKFZp434M229 (from clone DKFZp434M229)
536	AA985007		Homo sapiens mRNA; cDNA DKFZp564A026 (from clone DKFZp564A026)
537	AA938345		Homo sapiens mRNA; cDNA DKFZp564N1116 (from clone DKFZp564N1116)
538	AA129758		Homo sapiens mRNA; cDNA DKFZp761K2024 (from clone DKFZp761K2024)
539	AI276126		Human DNA sequence from clone RP4-756G23 on chromosome 22q13.313.33
540	AI301241		ESTs, Highly similar to AF172268 1 Traf2 and NCK interacting kinase, splice variant 5
541	AI291118		ESTs, Highly similar to AF219140 1 gastric cancer-related protein GCYS-20 [H.sapiens]
542	AA143060		ESTs, Highly similar to I38945 melanoma ubiquitous mutated protein [H.sapiens]
543	AI304351		ESTs, Moderately similar to NFY-C [H.sapiens]
544	AA923049		ESTs, Weakly similar to cytokine receptor-like factor 2; cytokine receptor CRL2 precusor
545	AA604003		ESTs, Weakly similar to CTL1 protein [H.sapiens]
546	AA847242		ESTs, Weakly similar to G786_HUMAN PROTEIN GS3786 [H.sapiens]
547	AI274179		ESTs, Weakly similar to LIV protein [H.sapiens]
548	R87741		ESTs, Weakly similar to RAB8_HUMAN RAS-RELATED PROTEIN RAB-8 [H.sapiens]
549	AA465193		ESTs, Weakly similar to unnamed protein product [H.sapiens]
550	AI266124		ESTs, Weakly similar to unnamed protein product [H.sapiens]
551	AA777360	*KIAA1002*	ESTs
552	AA358397		ESTs

553	AA129817	ESTs
554	F06091	ESTs
555	H42099	ESTs
556	AI090386	ESTs
557	AA449335	ESTs
558	AI243456	ESTs
559	AI355928	ESTs
560	R45502	ESTs
561	AA630642	ESTs
562	AA781393	ESTs
563	AA528243	ESTs
564	AA430699	ESTs
565	AA528190	ESTs
566	AA369905	ESTs
567	AI201894	ESTs
568	AI342469	ESTs
569	AA313152	ESTs
570	AI299327	ESTs
571	AI341332	ESTs
572	N33189	ESTs
573	W37776	ESTs
574	AI023557	ESTs
575	AA418448	ESTs
576	AA458558	ESTs
577	H52704	ESTs
578	AA142875	ESTs
579	AI366443	ESTs
580	H96559	ESTs
581	H98777	ESTs
582	AA989233	ESTs
583	AI032354	ESTs
584	W93000	ESTs
585	AA446184	ESTs
586	AI291207	ESTs
587	AA699359	ESTs
588	AA447217	ESTs
589	AA769604	ESTs

590	AI208970	ESTs
591	N93057	ESTs
592	AI225224	ESTs
593	W67193	ESTs
594	AI022649	ESTs
595	AA625553	ESTs
596	AA446064	ESTs
597	D61466	ESTs
598	H05777	ESTs
599	N30923	ESTs
600	AA135406	ESTs
601	AA661636	ESTs
602	H98796	ESTs
603	AI927063	ESTs
604	AA687594	ESTs
605	AA879280	ESTs

Table 5 Representative up-regulated genes with known function in pancreatic cancers

PNC Assignment	Accession No.	Symbol	Gene Name
genes involved in signal transduction pathway			
12	AA916826	*APP*	amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer disease)
13	L20688	*ARHGDIB*	Rho GDP dissociation inhibitor (GDI) beta
59	L36645	*EPHA4*	EphA4
69	J03260	*GNAZ*	guanine nucleotide binding protein (G protein), alpha z polypeptide
100	AA574178	*KAI1*	Kangai 1
119	AA458825	*MTIF2*	mitochondrial translational initiation factor 2
130	M80482	*PACE4*	paired basic amino acid cleaving system 4
135	M16750	*PIM1*	pim oncogene
151	X62006	*PTB*	polypyrimidine tract binding protein (heterogeneous nuclear ribonucleoprotein I)
154	AA346311	*RAI3*	retinoic acid induced 3
156	S45545	*RCV1*	recoverin
163	D38583	*S100A11*	S100 calcium-binding protein A11 (calgizzarin)
164	AA308062	*S100P*	S100 calcium-binding protein P
169	AF029082	*SFN*	stratifin
177	J03040	*SPARC*	secreted protein, acidic, cysteine-rich (osteonectin)

transcriptional factors			
8	AF047002	*ALY*	transcriptional coactivator
44	AA905901	*CRSP3*	cofactor required for Sp1 transcriptional activation, subunit 3 (130kD)
63	L16783	*FOXM1*	forkhead box M1
66	AA418167	*GATA3*	GATA-binding protein 3
80	X92518	*HMGIC*	high-mobility group (nonhistone chromosomal) protein isoform I-C
83	M16937	*HOXB7*	homeo box B7
108	U24576	*LMO4*	LIM domain only 4
120	X13293	*MYBL2*	v-myb avian myeloblastosis viral oncogene homolog-like 2
155	X64652	*RBMS1*	RNA binding motif, single stranded interacting protein 1
180	M81601	*TCEA1*	transcription elongation factor A (SII), 1
cell adhesion and cytoskeleton			
14	AF006086	*ARPC3*	actin related protein 2/3 complex, subunit 3 (21 kD)
28	AA557142	*CD2AP*	CD2-associated protein
32	X63629	*CDH3*	cadherin 3, type 1, P-cadherin (placental)
38	AA977821	*COL1A1*	collagen, type I, alpha 1
39	J03464	*COL1A2*	collagen, type I, alpha 2
40	X14420	*COL3A1*	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)
41	AI140851	*COL6A1*	collagen, type VI, alpha 1
46	U16306	*CSPG2*	chondroitin sulfate proteoglycan 2 (versican)
62	X02761	*FN1*	fibronectin 1
98	M15395	*ITGB2*	integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac) beta subunit)
104	J00269	*KRT6A*	keratin 6A
131	L11370	*PCDH1*	protocadherin 1 (cadherin-like 1)
136	AA234962	*PKP3*	plakophilin 3
cell cycle			
35	X54941	*CKS1*	CDC28 protein kinase 1
63	L16783	*FOXM1*	forkhead box M1
102	U63743	*KNSL6*	kinesin-like 6 (mitotic centromere-associated kinesin)
143	AF044588	*PRC1*	protein regulator of cytokinesis 1
184	K02581	*TK1*	thymidine kinase 1, soluble

Comparison of clinicopathological parameters with the expression profiles indicated that altered expression of 76 genes was associated with lymph-node metastasis and

that of 168 genes with liver metastasis. In addition, expression levels of 84 genes were related to the recurrence of disease. These genome-wide expression profiles should provide useful information for finding candidate genes whose products might serve as specific tumor markers and/or as molecular targets for treatment of patients with pancreatic cancer.

Materials and Methods

Identification of genes responsible for clinicopathological data

Genes associated with clinicopathological features, such as lymph-node-positive (r) and –negative (n), liver metastasis-positive (r) and –negative (n), and early-recurrence (r) and late-recurrence (n), were chosen according to the these two criteria; (i) signal intensities are higher than the cut-off value in at least 80% of the cases; (ii) $| Med_r - Med_n |>=0.5$, where Med indicates the median derived from log-transformed relative expression ratios in two groups. Genes were selected as candidates when they met the criteria with a permutation p-value of smaller than 0.05 in each clinicopathological status.

First, we applied a random permutation test to identify genes that were expressed differently in following two groups. The mean (μ) and standard deviation (σ) were calculated from the log-transformed relative expression ratios of each gene in node-positive (r) and node-negative (n) cases, liver-metastasis-positive (r) and –negative (n), and early-recurrence (r) and late-recurrence (n), respectively. A discrimination score (DS) for each gene was defined as follows:

$$DS = (\mu_r - \mu_n) / (\sigma_r + \sigma_n)$$

We carried out permutation tests to estimate the ability of individual genes to distinguish with two groups; samples were randomly permutated between the two classes 10,000 times. Since the DS dataset of each gene showed a normal distribution, we calculated a P value for the user-defined grouping (Golub et al., 1999). For this analysis, we applied the expression data of 13 cases consisting of 4 lymph-node-positive and 9 negative cases, those of 11 cases consisting of 5 liver metastasis-positive and 6 negative cases, and those of 13 cases consisting of 7 early-recurrent cases and 6 late-recurrent cases. For these analyses were performed by using only StageIV cases according to UICC TNM classification.

Calculation of prediction score

We further calculated the prediction score of recurrence according to procedures described previously (Golub et al., 1999). Each gene (g_i) votes for either early-recurrent cases or late-recurrent cases depending on whether the expression level (x_i) in the sample is closer to the mean expression level of early-recurrent cases or late-recurrent cases in reference samples. The magnitude of the vote (v_i) reflects the deviation of the expression level in the sample from the average of the two classes:

$$V_i = | x_i - (\mu_r + \mu_n) /2 |$$

We summed the votes to obtain total votes for the early-recurrent cases (V_r) and late-recurrent cases (V_n), and calculated PS values as follows:

$$PS = ((V_r - V_n) / (V_r + V_n)) \times 100$$

reflecting the margin of victory in the direction of either early-recurrent cases or late-recurrent cases. PS values range from –100 to 100; a higher absolute value of PS reflects a stronger prediction.

Evaluation of classification and leave-one-out test

We calculated the classification score (CS) by using the prediction score of early-recurrent (PS_r) and late-recurrent cases (PS_n) in each gene set, as follows:

$$CS = (\mu_{PSr} - \mu_{PSn}) / (\sigma_{PSr} + \sigma_{PSn})$$

A larger value of CS indicates better separation of the two groups by the predictive-scoring system. For the leave-one-out test, one sample is withheld, the permutation p-value and mean expression levels are calculated using remaining samples, and the class of the withheld sample is subsequently evaluated by calculating its prediction score. We repeated this procedure for each of the 13 samples.

Results

Identification of genes correlated with clinicopathological features

Lymph-node metastasis and liver metastasis

In order to investigate relations between gene expression profiles and clinicopathological parameters, we searched genes that were possibly associated with lymph-node metastasis and liver metastasis that are important determining factors of patients' prognosis. We first examined the expression profiles and the status of lymph-node metastasis using nine lymph-node-positive and four node-negative cases, and identified 76 genes that

were associated with lymph node status by a random permutation (p-value <0.05) (Table 6). Of those, 35 genes were relatively up-regulated, and 41 genes were down-regulated in node-positive tumors (Figure 3) comparing with node-negative tumors as control. In addition, we compared expression profiles of 5 cases with predominant recurrence in liver with those of 6 cases with metastasis to other sites (local, peritoneal and chest). We identified 168 genes that showed altered expression patterns uniquely in cases that had liver metastasis (Table 7), and 60 of them were relatively up-regulated in tumors (Figure 4). These genes included some key factors which had been proposed to play crucial roles in tumor cell proliferation, invasion and metastasis: integrin, beta 4 (*ITGB4*) (Shaw et al., 1997), colony stimulating factor 1 (*CSF1*) (Chambers et al., 1997), basigin (*BSG*) (Guo et al., 2000), and kinesin-like 6 (*KNSL6*) (Scanlan MJ et al., 2002). Hierarchical clustering analysis using these identified gene sets was also able to clearly classify the groups with regard to lymph node status or those with liver metastasis, respectively (Figure 3, 4).

Prognosis

To further investigate genes that might be associated with prognosis, we compared expression profiles of 7 cases who had recurrence within 12 months after surgery (disease free interval <12 months; median 6.4 months) with those of 6 cases who had >12 months of disease free interval (median 17.0 months). As shown in Figure 5A, we identified 84 genes that were expressed differently between these two groups using a random permutation method ($p < 0.05$).

In attempt on establishment of a predictive scoring system using gene expression pattern for recurrence after surgery, we rank-ordered above prognostic 84 candidate genes on the basis of the magnitude of their permutation p-values (Table 8) and calculated the prediction score by the leave-one-out test for cross-validation using top 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80 and 84 genes on the rank-ordered list. To determine the number of discriminating genes giving the best separation of the two groups, we calculated a classification score (CS) for each gene set (Figure 5B). As show in Figure 5C, the best separation was obtained when we used 30 genes consisting of top 17 genes of up-regulated in late recurrence cases genes and top 13 genes of up-regulated in early recurrence cases genes in our candidate list for scores calculation.

Discussion

Pancreatic cancer is characterized by very aggressive progression and rapid recurrence after surgical treatment. It has been reported that the cumulative 1-, 3-, and 5-year disease free survival rate were 66%, 7%, and 3% respectively, and median disease-free survival time was the only 8 months (Sperti et al., 1997). Most common recurrent sites are the local region and the liver, and distant metastases appear in the peritoneal cavity. However, since the relationships between tumor characteristics and the recurrence patterns are still little understood, we compared the expression profiles to lymph-node status or liver metastasis. We identified 76 genes that might be associated with lymph-node status, and 168 genes with liver metastasis. These genes included some key molecules whose possible roles in tumor progression had been reported previously; *ITGB4* and *BSG* were up-regulated in lymph-node positive cases, and *KNSL6* and *KRT8* were relatively up-regulated in liver metastasis cases. *ITGB4* was reported to promote carcinoma invasion through a preferential and localized targeting of phosphoinositide-3 OH kinase activity (Shaw et al., 1997), supporting the possible involvement of *ITGB4* in lymph-node metastasis. *KNSL6,* a member of the kinesin family of motor proteins, is known to be involved in chromosome segregation during mitosis (Maney T et al., 1998). The transcript of *KNSL6* was highly expressed in colon cancer, and was identified as cancer antigens associated with a cancer-related serum IgG response (Scanlan MJ et al., 2002). Thus, this antigen could be a biological marker for diagnosis and for monitoring of recurrence site.

In addition, we identified 84 genes possibly associated with tumor recurrence of pancreatic cancers. Expression levels of a subset of 30 genes selected from these 84 genes would be useful for predicting the disease free interval after surgical operation (Figure 5). These results might be useful for selection of patients for active adjuvant therapy although larger-scale study will be required to further evaluate our prediction system.

Table6 A list of 76 Candidate Genes for lymph-node metastasis

PNC Assign ment	GenBank ID	Symbol	Gene Name
UP-REGULATED GENES			
606	D16480	*HADHA*	hydroxyacy dehydrogenase, subunitA
607	AF015767	*BRE*	brain and reproductive organ-expressed (TNFRSF1A modulator)
608	D49742	*HABP2*	hyaluronan-binding protein 2
609	M37400	*GOT1*	glutamic-oxaloacetic transaminase 1
610	Z11502	*ANXA13*	annexin A13
611	D32050	*AARS*	alanyl-tRNA synthetase
612	U42376	*LY6E*	lymphocyte antigen 6 complex, locus E

613	U68019	*MADH3*	MAD (mothers against decapentaplegic, Drosophila) homolog 3
614	AI248620	*AP3D1*	adaptor-related protein complex 3, delta 1 subunit
615	U24183	*PFKM*	phosphofructokinase, muscle
616	AA193416		ESTs
617	AA911109	*FLJ20254*	hypothetical protein FLJ20254
618	AF070616	*HPCAL1*	hippocalcin-like 1
619	AI143127		Dynactin 4
620	AA412250	*PYGB*	phosphorylase, glycogen; brain
621	D45131	*BSG*	basigin
622	AB010427	*WDR1*	WD repeat domain 1
623	H20386	*MYG1*	MYG1 protein
624	AA371593	*GCN1L1*	GCN1 (general control of amino-acid synthesis 1, yeast)-like 1
625	L31581	*CCR7*	chemokine (C-C motif) receptor 7
626	AA922357		DKFZp586A0618
627	U07424	*FARSL*	phenylalanine-tRNA synthetase-like
628	AI248327		FLJ22233
629	AF055022	*DKFZP727 M231*	DKFZP727M231 protein
630	M37435	*CSF1*	colony stimulating factor 1 (macrophage)
631	U34683	*GSS*	glutathione synthetase
632	L41351	*PRSS8*	protease, serine, 8 (prostasin)
633	X52186	*ITGB4*	integrin, beta 4
634	R52161		DKFZp434A2410
635	U23028	*EIF2B5*	eukaryotic translation initiation factor 2B, subunit 5 (epsilon, 82kD)
636	AI336230	*RPS8*	ribosomal protein S8
637	AI268861		EST
638	U73036	*IRF7*	interferon regulatory factor 7
639	AI097058		FLJ23538
640	L36151	*PIK4CA*	phosphatidylinositol 4-kinase, catalytic, alpha polypeptide
DOWN-REGULATED GENES			
641	AA747290	*RPS15A*	ribosomal protein S15a
642	AA641744	*RPA2*	replication protein A2 (32kD)
643	AI188196	*USP22*	ubiquitin specific protease 22
644	AI222007		ESTs
645	AA192445	*TMEPAI*	transmembrane, prostate androgen induced RNA
646	AW069055	*FLJ10773*	Likely ortholog of mouse NPC derived proline rich protein 1
647	AI365733		ESTs
648	AF017418	*MEIS2*	Meis (mouse) homolog 2
649	AF024714	*AIM2*	absent in melanoma 2
650	AU155489	*MMP7*	matrix metalloproteinase 7 (matrilysin, uterine)
651	AW779142	*HUMAGCG B*	chromosome 3p21.1 gene sequence
652	AA487669	*GSTM1*	glutathione S-transferase M1
653	AA601564	*DLG5*	discs, large (Drosophila) homolog 5
654	AI042204	*FLJ12895*	hypothetical protein FLJ12895
655	D14662	*KIAA0106*	anti-oxidant protein 2
656	BF059178	*NONO*	non-POU-domain-containing, octamer-binding
657	U70063	*ASAH*	N-acylsphingosine amidohydrolase (acid ceramidase)
658	AA091553	*UBE2H*	ubiquitin-conjugating enzyme E2H (homologous to yeast UBC8)
659	L12350	*THBS2*	thrombospondin 2
660	AA324335	*ERF.*	Ets2 repressor factor

661	AI626007	*NTRK1*	neurotrophic tyrosine kinase, receptor, type 1
662	AI261382	*SH120*	putative G-protein coupled receptor
663	AF046024	*UBE1C*	ubiquitin-activating enzyme E1C (homologous to yeast UBA3)
664	AI299911	*PPP3CA*	protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform
665	X07979	*ITGB1*	integrin, beta 1
666	W45244	*C3*	complement component 3
667	AI245516		EST
668	AA907519	*C3ORF4*	chromosome 3 open reading frame 4
669	D42041	*KIAA0088*	KIAA0088 protein
670	AI300002	*CCNI*	cyclin I
671	AI338165	*HEF1*	enhancer of filamentation 1 (cas-like docking; Crk-associated substrate related)
672	AI312689	*HE1*	epididymal secretory protein (19.5kD)
673	NM_006077	*CBARA1*	calcium binding atopy-related autoantigen 1
674	AF131847	*MRG15*	MORF-related gene 15
675	AA676585	*NPM1*	nucleophosmin (nucleolar phosphoprotein B23, numatrin)
676	U85658	*TFAP2C*	transcription factor AP-2 gamma (activating enhancer-binding protein 2 gamma)
677	AB011090	*KIAA0518*	Max-interacting protein
678	U93867	*RPC62*	polymerase (RNA) III (DNA directed) (62kD)
679	Z11531	*EEF1G*	eukaryotic translation elongation factor 1 gamma
680	AA676322	*MTF1*	metal-regulatory transcription factor 1
681	AI339006		DKFZp586L1121

Table7 A list of 168 Candidate Genes for liver metastasis

PNC Assignment	GenBank ID	Symbol	Gene Name
UP-REGULATED GENES			
682	U63743	*KNSL6*	kinesin-like 6 (mitotic centromere-associated kinesin)
683	U12707	*WAS*	Wiskott-Aldrich syndrome (eczema-thrombocytopenia)
684	AA904028	*PAPPA*	pregnancy-associated plasma protein A
685	T69711		EST
686	AI338282	*TIGA1*	Homo sapiens mRNA; cDNA DKFZp566L203 (from clone DKFZp566L203)
687	AA843756	*ID2*	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
688	AF076483	*PGLYRP*	peptidoglycan recognition protein
689	AA447852	*PC326*	PC326 protein
690	L13939	*AP1B1*	adaptor-related protein complex 1, beta 1 subunit
691	AI344213	*CCS*	copper chaperone for superoxide dismutase
692	X74929	*KRT8*	keratin 8
693	U92459	*GRM8*	glutamate receptor, metabotropic 8
694	AA078295		ESTs
695	AA084871	*YKT6*	SNARE protein
696	M26252	*PKM2*	pyruvate kinase, muscle
697	AI280555	*KIAA0860*	KIAA0860 protein
698	U09278	*FAP*	fibroblast activation protein, alpha
699	AA989386		EST

700	U01184	*FLII*	flightless I (Drosophila) homolog
701	NM_016401	*HSPC138*	hypothetical protein
702	AW245101	*E2IG3*	putative nucleotide binding protein, estradiol-induced
703	U47025	*PYGB*	phosphorylase, glycogen; brain
704	Z21507	*EEF1D*	eukaryotic translation elongation factor 1 delta
705	U38320	*MMP19*	matrix metalloproteinase 19
706	AA233644	*PPP1CC*	protein phosphatase 1, catalytic subunit, gamma isoform
707	L40401	*ZAP128*	peroxisomal long-chain acyl-coA thioesterase ; putative protein
708	AI365683		Homo sapiens PAC clone RP4-751H13 from 7q35-qter
709	AF039690	*SDCCAG8*	serologically defined colon cancer antigen 8
710	L19067	*RELA*	v-rel avian reticuloendotheliosis viral oncogene homolog A
711	U48734	*ACTN4*	actinin, alpha 4
712	M22324	*ANPEP*	alanyl (membrane) aminopeptidase
713	AA921921	*KIAA0414*	KIAA0414 protein
714	X97630	*EMK1*	ELKL motif kinase
715	AJ002308	*SYNGR2*	synaptogyrin 2
716	AA447019	*MAN1B1*	mannosidase, alpha, class 1B, member 1
717	M98252	*PLOD*	procollagen-lysine, 2-oxoglutarate 5-dioxygenase
718	H48649	*FGG*	fibrinogen, gamma polypeptide
719	AI139231	*FBL*	fibrillarin
720	AA249454		ESTs, Weakly similar to KIAA0227 [H.sapiens]
721	U89278	*EDR2*	early development regulator 2 (homolog of polyhomeotic 2)
722	M24398	*PTMS*	parathymosin
723	L41668	*GALE*	galactose-4-epimerase, UDP-
724	D78298	*VLCAD*	very-long-chain acyl-CoA dehydrogenase
725	X89602	*HSRTSBETA*	rTS beta protein
726	M91029	*AMPD2*	adenosine monophosphate deaminase 2 (isoform L)
727	X73478	*PPP2R4*	protein phosphatase 2A, regulatory subunit B' (PR 53)
728	AI189477	*IDH2*	isocitrate dehydrogenase 2 (NADP+), mitochondrial
729	D30612	*ZNF282*	zinc finger protein 282
730	AA506972	*KIAA0668*	KIAA0668 protein
731	AA404724	*GPRK7*	G protein-coupled receptor kinase 7
732	AB001451	*SLI*	neuronal Shc adaptor homolog
733	AL120683	*LASS2*	LAG1 longevity assurance homolog 2 (S. cerevisiae)
734	H20386	*MYG1*	MYG1 protein
735	AA477862	*KIAA0974*	KIAA0974 protein
736	AF075590	*BZRP*	benzodiazapine receptor (peripheral)
737	AA748421	*TFR2*	transferrin receptor 2
738	AA639771	*MMP12*	matrix metalloproteinase 12 (macrophage elastase)
739	AI218495		ESTs, Moderately similar to integral inner nuclear membrane protein MAN1
740	N80334	*DKFZP586O0223*	hypothetical protein
741	AA847660	*HEXA*	hexosaminidase A (alpha polypeptide)
DOWN-REGULATED GENES			
742	S74678	*HNRPK*	heterogeneous nuclear ribonucleoprotein K
743	D56784	*DEK*	DEK oncogene (DNA binding)
744	U31383	*GNG10*	guanine nucleotide binding protein 10
745	H06970	*STK24*	serine/threonine kinase 24 (Ste20, yeast homolog)

746	AF038954	*ATP6J*	ATPase, H+ transporting, lysosomal (vacuolar proton pump), member J
747	W19984	*DREV1*	CGI-81 protein
748	AA282650	*SAC1*	Suppressor of actin 1
749	U16738	*RPL14*	ribosomal protein L14
750	AA614311	*VCP*	valosin-containing protein
751	AF006088	*ARPC5*	actin related protein 2/3 complex, subunit 5 (16 kD)
752	AF007871	*DYT1*	dystonia 1, torsion (autosomal dominant; torsin A)
753	D21090	*RAD23B*	RAD23 (S. cerevisiae) homolog B
754	AA910279	*STAU*	staufen (Drosophila, RNA-binding protein)
755	AA226073	*ITM2C*	integral membrane protein 2C
756	AA583455	*RNF7*	ring finger protein 7
757	AA731151	*KIAA1085*	KIAA1085 protein
758	U14575	*PPP1R8*	protein phosphatase 1, regulatory (inhibitor) subunit 8
759	M81637	*GCL*	grancalcin
760	L37368	*RNPS1*	RNA-binding protein S1, serine-rich domain
761	AK000403	*FLJ20396*	hypothetical protein FLJ20396
762	D13315	*GLO1*	glyoxalase I
763	U66818	*UBE2I*	ubiquitin-conjugating enzyme E2I (homologous to yeast UBC9)
764	X56351	*ALAS1*	aminolevulinate, delta-, synthase 1
765	L08424	*ASCL1*	achaete-scute complex (Drosophila) homolog-like 1
766	X15187	*TRA1*	tumor rejection antigen (gp96) 1
767	U33286	*CSE1L*	chromosome segregation 1 (yeast homolog)-like
768	AA747290	*RPS15A*	ribosomal protein S15a
769	AI148832	*KIAA1209*	KIAA1209 protein
770	S65738	*ADF*	destrin (actin depolymerizing factor)
771	X53586	*ITGA6*	integrin, alpha 6
772	U31906	*GOLGA4*	golgi autoantigen, golgin subfamily a, 4
773	AA664213	*DKC1*	dyskeratosis congenita 1, dyskerin
774	AI338165	*HEF1*	enhancer of filamentation 1 (cas-like docking; Crk-associated substrate related)
775	W74416	*LOC51126*	N-terminal acetyltransferase complex ard1subunit
776	AI125978	*SNX2*	sorting nexin 2
777	H96478		EST
778	U46570	*TTC1*	tetratricopeptide repeat domain 1
779	U21242	*GTF2A2*	general transcription factor IIA, 2 (12kD subunit)
780	W95089	*HSPC033*	HSPC033 protein
781	D55654	*MDH1*	malate dehydrogenase 1, NAD (soluble)
782	AF072860	*PRKRA*	protein kinase, interferon-inducible double stranded RNA dependent activator
783	AF042081	*SH3BGRL*	SH3 domain binding glutamic acid-rich protein like
784	D63881	*KIAA0160*	KIAA0160 protein
785	AA195740		Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 41832
786	M36341	*ARF4*	ADP-ribosylation factor 4
787	C06051	*JAK1*	Janus kinase 1 (a protein tyrosine kinase)
788	D28473	*IARS*	isoleucine-tRNA synthetase
789	R23830		ESTs
790	U51166	*TDG*	thymine-DNA glycosylase
791	AA128470	*DSP*	desmoplakin (DPI, DPII)
792	M77698	*YY1*	YY1 transcription factor
793	AI272932	*BAG5*	BCL2-associated athanogene 5

794	U45879	*BIRC2*	baculoviral IAP repeat-containing 2
795	Z35491	*BAG1*	BCL2-associated athanogene
796	AF016507	*CTBP2*	C-terminal binding protein 2
797	X89478	*HRB*	HIV Rev binding protein
798	X06323	*MRPL3*	mitochondrial ribosomal protein L3
799	M29065	*HNRPA2B1*	heterogeneous nuclear ribonucleoprotein A2/B1
800	AA431846	*LOC51187*	60S ribosomal protein L30 isolog
801	E02628		polypeptide chain elongation factor 1 alpha
802	AI349804		EST
803	X99584	*SMT3H1*	SMT3 (suppressor of mif two 3, yeast) homolog 1
804	D13630	*KIAA0005*	KIAA0005 gene product
805	U24223	*PCBP1*	poly(rC)-binding protein 1
806	AA315729		FLJ23197
807	AA401318	*DKFZP566 D193*	DKFZP566D193 protein
808	AA524350	*LOC51719*	MO25 protein
809	AB004857	*SLC11A2*	solute carrier family 11, member 2
810	AA379042	*PUM2*	Pumilio (Drosophila) homolog 2
811	AW779142	*HUMAGCGB*	chromosome 3p21.1 gene sequence
812	R39044		Homo sapiens clone 25194 mRNA sequence
813	M58458	*RPS4X*	ribosomal protein S4, X-linked
814	H89110		ESTs
815	U47077	*PRKDC*	protein kinase, DNA-activated, catalytic polypeptide
816	AA236252	*ASH2L*	ash2 (absent, small, or homeotic, Drosophila, homolog)-like
817	D50683	*TGFBR2*	transforming growth factor, beta receptor II (70-80kD)
818	M61199	*SSFA2*	sperm specific antigen 2
819	U56637	*CAPZA1*	capping protein (actin filament) muscle Z-line, alpha 1
820	AA514818	*KIAA0068*	KIAA0068 protein
821	N45298	*ARHGEF12*	Rho guanine exchange factor (GEF) 12
822	X76104	*DAPK1*	death-associated protein kinase 1
823	D14812	*KIAA0026*	MORF-related gene X
824	AA357508		Homo sapiens clone 24711 mRNA sequence
825	U96915	*SAP18*	sin3-associated polypeptide, 18kD
826	D10522	*MACS*	myristoylated alanine-rich protein kinase C substrate (MARCKS, 80K-L)
827	N46856		Homo sapiens cDNA: FLJ23091 fis, clone LNG07220
828	D26125	*AKR1C4*	aldo-keto reductase family 1, member C4
829	AI085802	*CAV2*	Caveolin 2
830	AI289407	*ZNF207*	zinc finger protein 207
831	U54831	*TOP2B*	topoisomerase (DNA) II beta (180kD)
832	AA281115	*UBQLN1*	ubiquilin 1
833	N41902	*CLTH*	Clathrin assembly lymphoid-myeloid leukemia gene
834	AA432312	*TSPYL*	TSPY-like
835	AF006516	*SSH3BP1*	spectrin SH3 domain binding protein 1
836	AA706503	*EEF1A1*	eukaryotic translation elongation factor 1 alpha 1
837	N95414		ESTs
838	M20472	*CLTA*	clathrin, light polypeptide (Lca)
839	AI078833	*TAX1BP1*	Tax1 (human T-cell leukemia virus type I) binding protein 1
840	U09953	*RPL9*	ribosomal protein L9
841	U44772	*PPT1*	palmitoyl-protein thioesterase 1
842	AA973853		Homo sapiens cDNA FLJ20532 fis, clone KAT10877

843	U81504	*AP3B1*	adaptor-related protein complex 3, beta 1 subunit
844	AA634090	*HNRPA1*	heterogeneous nuclear ribonucleoprotein A1
845	U83463	*SDCBP*	syndecan binding protein (syntenin)
846	AI092703	*FBXW1B*	f-box and WD-40 domain protein 1B
847	AF052113	*Rab14*	GTPase Rab14
848	AF007216	*SLC4A4*	solute carrier family 4, sodium bicarbonate cotransporter, member 4
849	AA809819	*CREG*	cellular repressor of E1A-stimulated genes

Table8 A list of 84 Candidate Genes for prognosis

PNC Assignment	GenBank ID	Symbol	Gene Name
up-regulated in late recurrence cases			
850	AF049884	*ARGBP2*	Arg/Abl-interacting protein ArgBP2
851	NM_006077	*CBARA1*	calcium binding atopy-related autoantigen 1
852	Z11531	*EEF1G*	eukaryotic translation elongation factor 1 gamma
853	AW157203	*LCAT*	lecithin-cholesterol acyltransferase
854	AI123363	*RPL23A*	ribosomal protein L23a
855	X53777	*RPL17*	ribosomal protein L17
856	U16798	*ATP1A1*	ATPase, Na+/K+ transporting, alpha 1 polypeptide
857	X76013	*QARS*	glutaminyl-tRNA synthetase
858	AF075590	*BZRP*	benzodiazapine receptor (peripheral)
859	L38995	*TUFM*	Tu translation elongation factor, mitochondrial
860	H89783	*SERPINA4*	serine (or cysteine) proteinase inhibitor, clade A , member 4
861	D83782	*SCAP*	SREBP CLEAVAGE-ACTIVATING PROTEIN
862	M75126	*HK1*	hexokinase 1
863	AA936173	*RPS11*	ribosomal protein S11
864	AA488766	*SYNGR2*	synaptogyrin 2
865	M60922	*FLOT2*	flotillin 2
866	D26600	*PSMB4*	proteasome (prosome, macropain) subunit, beta type, 4
867	L19711	*DAG1*	dystroglycan 1 (dystrophin-associated glycoprotein 1)
868	AI148194		Novel human gene mapping to chomosome 22
869	X57398	*PM5*	pM5 protein
870	M17886	*RPLP1*	ribosomal protein, large, P1
871	L14778	*PPP3CA*	protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform (calcineurin A alpha)
872	AA156481	*RPL13A*	ribosomal protein L13a
873	AA083406	*EIF3S8*	eukaryotic translation initiation factor 3, subunit 8 (110kD)
874	AF000984	*DBY*	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide, Y chromosome
875	X17206	*RPS2*	ribosomal protein S2
876	W45522	*LOC51189*	ATPase inhibitor precursor
877	X83218	*ATP5O*	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit
878	AI246699	*CATX-8*	CATX-8 protein
879	AA029875	*CASP4*	caspase 4, apoptosis-related cysteine protease
880	AI366139	*MAC30*	hypothetical protein
881	U46191	*RAGE*	renal tumor antigen

882	AA487669	*GSTM1*	glutathione S-transferase M1
883	AI131289	*RPLP2*	ribosomal protein, large P2
884	AI299327		ESTs
885	AA922716	*PRKACB*	protein kinase, cAMP-dependent, catalytic, beta
886	AA845165	*PRSS1*	protease, serine, 1 (trypsin 1)
887	AA877534	*GPRC5C*	G protein-coupled receptor, family C, group 5, member C
888	C01335		ESTs, Weakly similar to FLDED [H.sapiens]
889	Z26876	*RPL38*	ribosomal protein L38
890	AI080640	*AGR2*	anterior gradient 2 (Xenepus laevis) homolog
891	X04588	*TPM3*	2.5kb mRNA for cytoskeletal tropomyosin TM30
892	D30949		Homo sapiens cDNA FLJ12750 fis, clone NT2RP2001168, weakly similar to VERPROLIN
893	Z11559	*ACO1*	aconitase 1, soluble
up-regulated in early recurrence cases			
894	AA700379	*MTMR1*	myotubularin related protein 1
895	AI340331	*HT010*	uncharacterized hypothalamus protein HT010
896	AA459167	*NPD002*	NPD002 protein
897	AI014395	*YME1L1*	YME1 (S.cerevisiae)-like 1
898	M94083	*CCT6A*	chaperonin containing TCP1, subunit 6A (zeta 1)
899	M22382	*HSPD1*	heat shock 60kD protein 1 (chaperonin)
900	AA150867	*TIMM9*	translocase of inner mitochondrial membrane 9 (yeast) homolog
901	L76687	*GRB14*	growth factor receptor-bound protein 14
902	T70782	*FLJ10803*	hypothetical protein FLJ10803
903	AI018632	*LAMP1*	lysosomal-associated membrane protein 1
904	AA531437	*MLLT4*	myeloid/lymphoid or mixed-lineage leukemia translocated to, 4
905	AI075048	*CTSB*	cathepsin B
906	AL031668	*RALY*	RNA-binding protein (autoantigenic)
907	AI357601	*RPL37A*	ribosomal protein L37a
908	U51586	*SIAHBP1*	siah binding protein 1
909	AF004430	*TPD52L2*	tumor protein D52-like 2
910	AI279562	*KIAA0469*	KIAA0469 gene product
911	M11717	*HSPA1A*	heat shock 70kD protein 1A
912	AF015767	*BRE*	brain and reproductive organ-expressed (TNFRSF1A modulator)
913	X06323	*MRPL3*	mitochondrial ribosomal protein L3
914	AI305234		ESTs
915	W24533	*GRB10*	growth factor receptor-bound protein 10
916	AA504081	*CSH2*	chorionic somatomammotropin hormone 2
917	AA778572	*HSPC164*	hypothetical protein
918	D11999	*GLS*	glutaminase
919	D32050	*AARS*	alanyl-tRNA synthetase
920	D63997	*GOLGA3*	golgi autoantigen, golgin subfamily a, 3
921	R64726		Homo sapiens cDNA: FLJ23591 fis, clone LNG14729
922	M61715	*WARS*	tryptophanyl-tRNA synthetase
923	AI090753	*SHMT2*	serine hydroxymethyltransferase 2 (mitochondrial)
924	AI289991	*DKFZP761C169*	hypothetical protein DKFZp761C169
925	AA345061	*KIAA0903*	KIAA0903 protein
926	AA255699		Human DNA sequence from clone RP3-324O17 on chromosome 20
927	H73961	*ARPC3*	actin related protein 2/3 complex, subunit 3 (21 kD)
928	D87666	*GPI*	glucose phosphate isomerase

929	AI075943	*SENP2*	sentrin-specific protease
930	D87989	*UGTREL1*	UDP-galactose transporter related
931	D86956	*HSP105B*	heat shock 105kD
932	L13740	*NR4A1*	nuclear receptor subfamily 4, group A, member 1
933	AA320379	*POH1*	26S proteasome-associated pad1 homolog

Industrial Applicability

The gene-expression analysis of pancreatic cancer described herein, obtained through a combination of laser-capture dissection and genome-wide cDNA microarray, has identified specific genes as targets for cancer prevention and therapy. Based on the expression of a subset of these differentially expressed genes, the present invention provides a molecular diagnostic markers for identifying or detecting pancreatic cancer.

The methods described herein are also useful in the identification of additional molecular targets for prevention, diagnosis and treatment of pancreatic cancer. The data reported herein add to a comprehensive understanding of pancreatic cancer, facilitate development of novel diagnostic strategies, and provide clues for identification of molecular targets for therapeutic drugs and preventative agents. Such information contributes to a more profound understanding of pancreatic tumorigenesis, and provide indicators for developing novel strategies for diagnosis, treatment, and ultimately prevention of pancreatic cancer.

All patents, patent applications, and publications cited herein are incorporated by reference in their entirety. Furthermore, while the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope of the invention.

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CLAIMS

1. A method of diagnosing PNC or a predisposition to developing PNC in a subject, comprising determining a level of expression of a PNC-associated gene in a patient derived biological sample, wherein an increase or decrease of said level compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing PNC.

2. The method of claim 1, wherein said PNC-associated gene is selected from the group consisting of PNC 1-259, wherein an increase in said level compared to a normal control level indicates said subject suffers from or is at risk of developing PNC.

3. The method of claim 1, wherein said increase is at least 10% greater than said normal control level.

4. The method of claim 1, wherein said PNC-associated gene is selected from the group consisting of PNC 260-605, wherein a decrease in said level compared to a normal control level indicates said subject suffers from or is at risk of developing PNC.

5. The method of claim 4, wherein said decrease is at least 10% lower than said normal control level.

6. The method of claim 1, wherein said method further comprises determining said level of expression of a plurality of PNC-associated genes.

7. The method of claim 1, wherein the expression level is determined by any one method select from the group consisting of:
(a) detecting the mRNA of the PNC-associated genes,
(b) detecting the protein encoded by the PNC-associated genes, and
(c) detecting the biological activity of the protein encoded by the PNC-associated genes.

8 The method of claim 1, wherein said hybridization step is carried out on a DNA array.

9 The method of claim 1, wherein said biological sample comprises an epithelial cell.

10 The method of claim 1, wherein said biological sample comprises a pancreatic ductal adenocarcinoma cell.

11 The method of claim 7 wherein said biological sample comprises an epithelial cell

from a pancreatic ductal adenocarcinoma.

12 A PNC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PNC 1-605.

13 A PNC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PNC 1-259.

14 A PNC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PNC 260-605.

15 A method of screening for a compound for treating or preventing pancreatic cancer, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a polynucleotide selected from the group consisting of PNC 1-605;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting a compound that binds to the polypeptide.

16. A method of screening for a compound for treating or preventing pancreatic cancer, said method comprising the steps of:

a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of PNC 1-605; and

b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of PNC 1-259, or erevates the expression level of one or more marker genes selected from the group consisting of PNC 260-605.

17. The method of claim 16, wherein said cell comprises a pancreatic cancer cell.

18. A method of screening for a compound for treating or preventing pancreatic cancer, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a polynucleotide selected from the group consisting of PNC 1-605;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide encoded by the polynucleotide selected from the group consisting of PNC 1-259 in comparison with the biological activity detected in the absence of the test compound,

or enhances the biological activity of the polypeptide encoded by the polynucleotide selected from the group consisting of PNC 260-605 in comparison with the biological activity detected in the absence of the test compound.

19. A method of screening for compound for treating or preventing pancreatic cancer, said method comprising the steps of:

a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of PNC 1-605

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of PNC 1-259 or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of PNC 260-605, as compared to a control.

20. A kit comprising a detection reagent which binds to two or more nucleic acid sequences selected from the group consisting of PNC 1-605 or polypeptides encoded thereby.

21. An array comprising two or more nucleic acids which bind to one or more nucleic acid sequences selected from the group consisting of PNC 1-605.

22. A method of treating or preventing pancreatic cancer in a subject comprising administering to said subject an antisense composition, said composition comprising a nucleotide sequence complementary to a coding sequence selected from the group consisting of PNC 1-259.

23. A method of treating or preventing pancreatic cancer in a subject comprising administering to said subject a siRNA composition, wherein said composition reduces the expression of a nucleic acid sequence selected from the group consisting of PNC 1-259.

24. A method for treating or preventing pancreatic cancer in a subject comprising the step

of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PNC 1-259.

25. A method of treating or preventing pancreatic cancer in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acide selected from the group consisting of PNC 1-259 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.

26. A method of treating or preventing pancreatic cancer in a subject comprising administering to said subject a compoud that increases the expression or activity of a polynucleotide selected from the group consisting of PNC 260-605

27. A method for treating or preventing pancreatic cancer in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claims 15-19.

28. A method of treating or preventing pancreatic cancer in a subject comprising administering to said subject a pharmaceutically effective amount of a polynucleotide select from the group consisting of PNC 260-605, or polypeptide encoded by thereof.

29. A composition for treating or preventing pancreatic cancer, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against a polynucleotide select from the group consisting of PNC 1-259.

30. A composition for treating or preventing pancreatic cancer, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PNC 1-259.

31. A composition for treating or preventing pancreatic cancer, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claims 15-19 as an active ingredient, and a pharmaceutically acceptable carrier.

32. A method of screening for a compound for treating or preventing malignant pancreatic cancer, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a polynucleotide selected from the group consisting of PNC 606-681 and PNC 682-849;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting a compound that binds to the polypeptide.

33. A method of screening for a compound for treating or preventing malignant pancreatic cancer, said method comprising the steps of:

a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes are selected from the group consisting of PNC 606-681 and PNC 682-849; and

b) selecting a compound that reduces the expression level of one or more up-regulated marker genes selected from the group consisting of PNC 606-640 and PNC 682-741, or elevates the expression level of one or more down-regulated marker genes selected from the group consisting of PNC 641-681 and PNC 742-849.

34. The method of claim 33, wherein said cell comprises a malignant pancreatic cancer cell.

35. A method of screening for a compound for treating or preventing malignant pancreatic cancer, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a polynucleotide selected from the group consisting of PNC 606-681 and PNC 682-849;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide encoded by an up-regulated marker gene selected from the group consisting of PNC 606-640 and PNC 682-741 in comparison with the biological activity detected in the absence of the test compound, or enhances the biological activity of the polypeptide encoded by a down-regulated marker gene selected from the group consisting of PNC 641-681 and PNC 742-849 in comparison with the biological activity detected in the absence of the test compound.

36. A method of screening for compound for treating or preventing malignant pancreatic cancer, said method comprising the steps of:

a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene

that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of PNC 606-681 and PNC 682-849;

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of PNC 606-640 and PNC 682-741 or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of PNC 641-681 and PNC 742-849, as compared to a control.

37. A method for treating or preventing malignant pancreatic cancer in a subject comprising administering to said subject an antisense composition, said composition comprising a nucleotide sequence complementary to a coding sequence of an up-regulated gene selected from the group consisting of PNC 606-640 and PNC 682-741.

38. A method for treating or preventing malignant pancreatic cancer in a subject comprising administering to said subject an siRNA composition, wherein said composition reduces the expression of a nucleic acid sequence of an up-regulated gene selected from the group consisting of PNC 606-640 and PNC 682-741.

39. A method for treating or preventing malignant pancreatic cancer in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one of up-regulated genes selected from the group consisting of PNC 606-640 and PNC 682-741.

40. A method for treating or preventing malignant pancreatic cancer in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by an up-regulated gene selected from the group consisting of PNC 606-640 and PNC 682-741 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.

41. A method for treating or preventing malignant pancreatic cancer in a subject comprising administering to said subject a compound that increases the expression or activity of a down-regulated gene selected from the group consisting of PNC 641-681

and PNC 742-849.

42. A method for treating or preventing malignant pancreatic cancer in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claims 32-36.

43. A method for treating or preventing malignant pancreatic cancer in a subject comprising administering to said subject a pharmaceutically effective amount of down-regulated gene selected from the group consisting of PNC 641-681 and PNC 742-849, or polypeptide encoded thereby.

44. A composition for treating or preventing malignant pancreatic cancer, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against an up-regulated gene selected from the group consisting of PNC 606-640 and PNC 682-741.

45. A composition for treating or preventing malignant pancreatic cancer, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one of up-regulated genes selected from the group consisting of PNC 606-640 and PNC 682-741.

46. A composition for treating or preventing malignant pancreatic cancer, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claims 32-36 as an active ingredient, and a pharmaceutically acceptable carrier.

47. A method of screening for a compound for treating or preventing recurrence of pancreatic cancer, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a polynucleotide selected from the group consisting of PNC 850-933;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting a compound that binds to the polypeptide.

48. A method of screening for a compound for treating or preventing recurrence of pancreatic cancer, said method comprising the steps of:

a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes are selected from the group consisting of

PNC 850-933; and

b) selecting a compound that reduces the expression level of one or more up-regulated marker genes selected from the group consisting of PNC 894-933, or elevates the expression level of one or more up-regulated marker genes in late recurrence cases selected from the group consisting of PNC 850-893.

49. The method of claim 48, wherein said cell comprises a recurrent pancreatic cancer cell.

50. A method of screening for a compound for treating or preventing recurrence of pancreatic cancer, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a polynucleotide selected from the group consisting of PNC 850-933;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide encoded by a marker gene selected from the group consisting of PNC 894-933 in comparison with the biological activity detected in the absence of the test compound, or enhances the biological activity of the polypeptide encoded by an up-regulated marker gene in late recurrence cases selected from the group consisting of 850-893 in comparison with the biological activity detected in the absence of the test compound.

51. A method of screening for a compound for treating or preventing recurrence of pancreatic cancer, said method comprising the steps of:

a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of PNC 850-933;

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of PNC 894-933 or that enhances the expression level of said reporter gene when said marker gene is a up-regulated marker gene in late recurrence cases selected from the group consisting of PNC 850-893, as compared to a control.

52. A method of treating or preventing recurrence of pancreatic cancer in a subject comprising administering to said subject an antisense composition, said composition comprising a nucleotide sequence complementary to a coding sequence of an up-regulated gene selected from the group consisting of PNC 894-933.

53. A method of treating or preventing recurrence of pancreatic cancer in a subject comprising administering to said subject an siRNA composition, wherein said composition reduces the expression of a nucleic acid sequence of an up-regulated gene selected from the group consisting of PNC 894-933.

54. A method for treating or preventing recurrence of pancreatic cancer in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one of up-regulated genes selected from the group consisting of PNC 894-933.

55. A method of treating or preventing recurrence of pancreatic cancer in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by an up-regulated gene selected from the group consisting of PNC 894-933 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.

56. A method of treating or preventing recurrence of pancreatic cancer in a subject comprising administering to said subject a compound that increases the expression or activity of a up-regulated gene in late recurrence cases selected from the group consisting of PNC 850-893.

57. A method for treating or preventing recurrence of pancreatic cancer in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claims 47-51.

58. A method of treating or preventing recurrence of pancreatic cancer in a subject comprising administering to said subject a pharmaceutically effective amount of a up-regulated gene in late recurrence cases selected from the group consisting of PNC 850-893, or polypeptide encoded thereby.

59. A composition for treating or preventing recurrence of pancreatic cancer, said composition comprising a pharmaceutically effective amount of an antisense

polynucleotide or small interfering RNA against an up-regulated gene selected from the group consisting of PNC 894-933.

60. A composition for treating or preventing recurrence of pancreatic cancer, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one of up-regulated genes selected from the group consisting of PNC 894-933.

61. A composition for treating or preventing recurrence of pancreatic cancer, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claims 47-51 as an active ingredient, and a pharmaceutically acceptable carrier.

62. A method of predicting recurrence of PNC, the method comprising the steps of:

(a) detecting an expression level of one or more marker genes in a specimen collected from a subject to be predicted, wherein the one or more marker genes are selected from the group consisting of PNC 850-866 (ARGBP2, CBARA1, EEF1G, LCAT, RPL23A, RPL17, ATP1A1, QARS, BZRP, TUFM, SERPINA4, SCAP, HK1, RPS11, SYNGR2, FLOT2, and PSMB4), 894-906 (MTMR1, HT010, NPD002, YME1L1, CCT6A, HSPD1, TIMM9, GRB14, FLJ10803, LAMP1, MLLT4, CTSB, RALY);

(b) comparing the expression level of the one or more marker genes to that of a early recurrence case and late recurrence case; and

(c) when the expression level of the one or more marker genes close to that of a early recurrence case, is indicative of risk of recurrence of PNC, or when the expression level of the one or more marker genes close to that of a late recurrence case, is indicative of low risk of recurrence of PNC.

63. The method of claim 62, wherein step (c) further comprises the steps of calculating a prediction score comprising following steps :

i) calculating the magnitude of the vote (Vi) by the following formula:

$$V_i = | x_i - (\mu_r + \mu_n) /2 |$$

in the fomula ; Xi is the expression level in the sample, μ_r is the expression level in the early recurrence case, and μ_n is the expression level in the late recurrence case,

ii) calculating PS values by following formula:

$$PS = ((V_r - V_n) / (V_r + V_n)) \times 100$$

in the fomula; V_r and V_n is the total vote of early-recurrent cases and late-recurrent, respectively, and

iii) when the PS values is more than 0, determining the subject to be at a risk of having recurrence of PNC and when the PS values is less than 0, determining the risk of the subject of having recurrence of PNC to be low.

64. A PNC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PNC 850-866, 894-906.

65. The expression profile of claim 64, wherein the gene expression is derived from from a pancreatic cancer cell of a patient with early recurrence or late recurrence.

66. A kit comprising a detection reagent which binds to two or more nucleic acid sequences selected from the group consisting of PNC 850-866, 894-906 or polypeptide encoded thereby.

67. An array comprising two or more nucleic acids which bind to one or more nucleic acid sequences selected from the group consisting of PNC 850-866, 894-906.

FIG.1

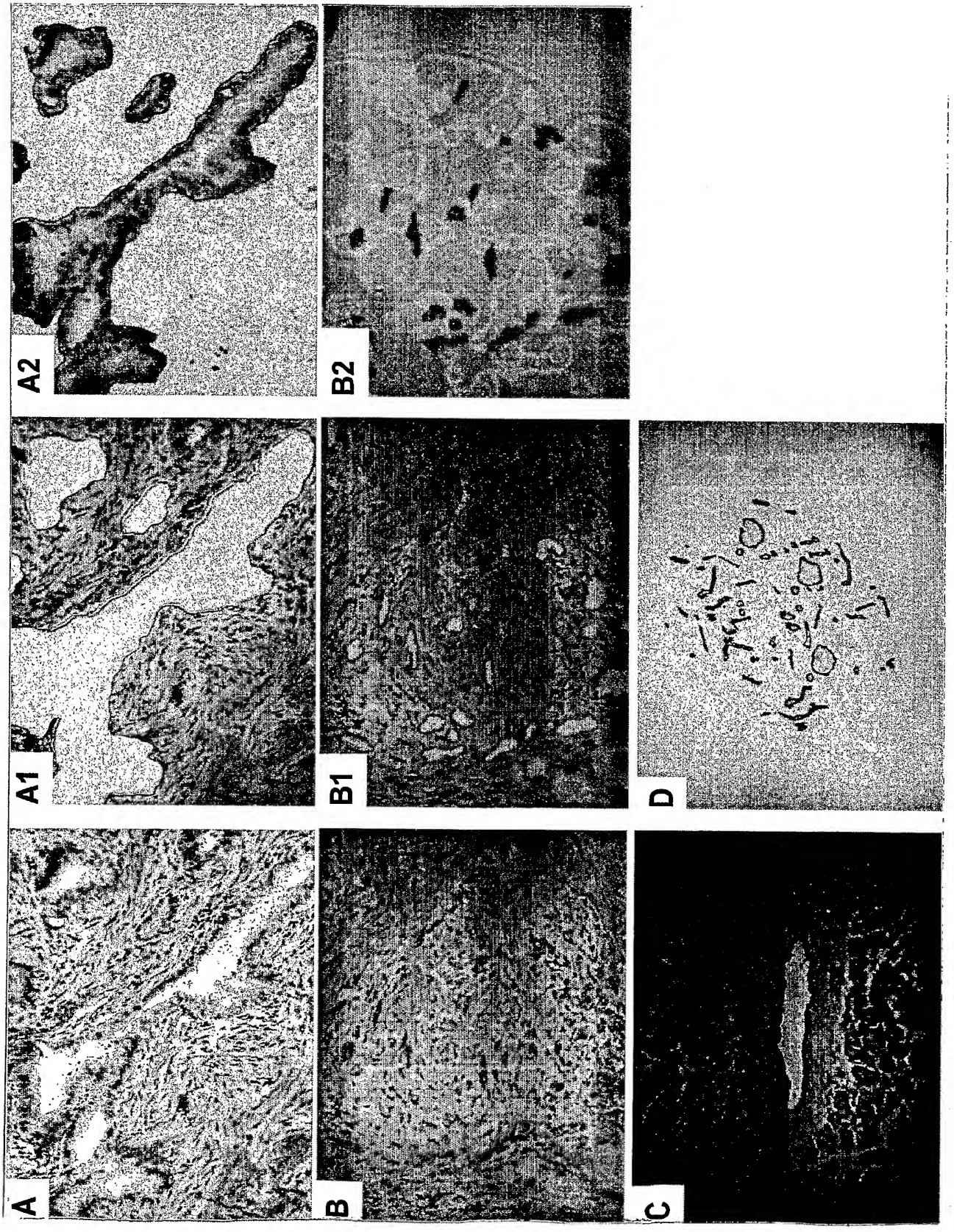
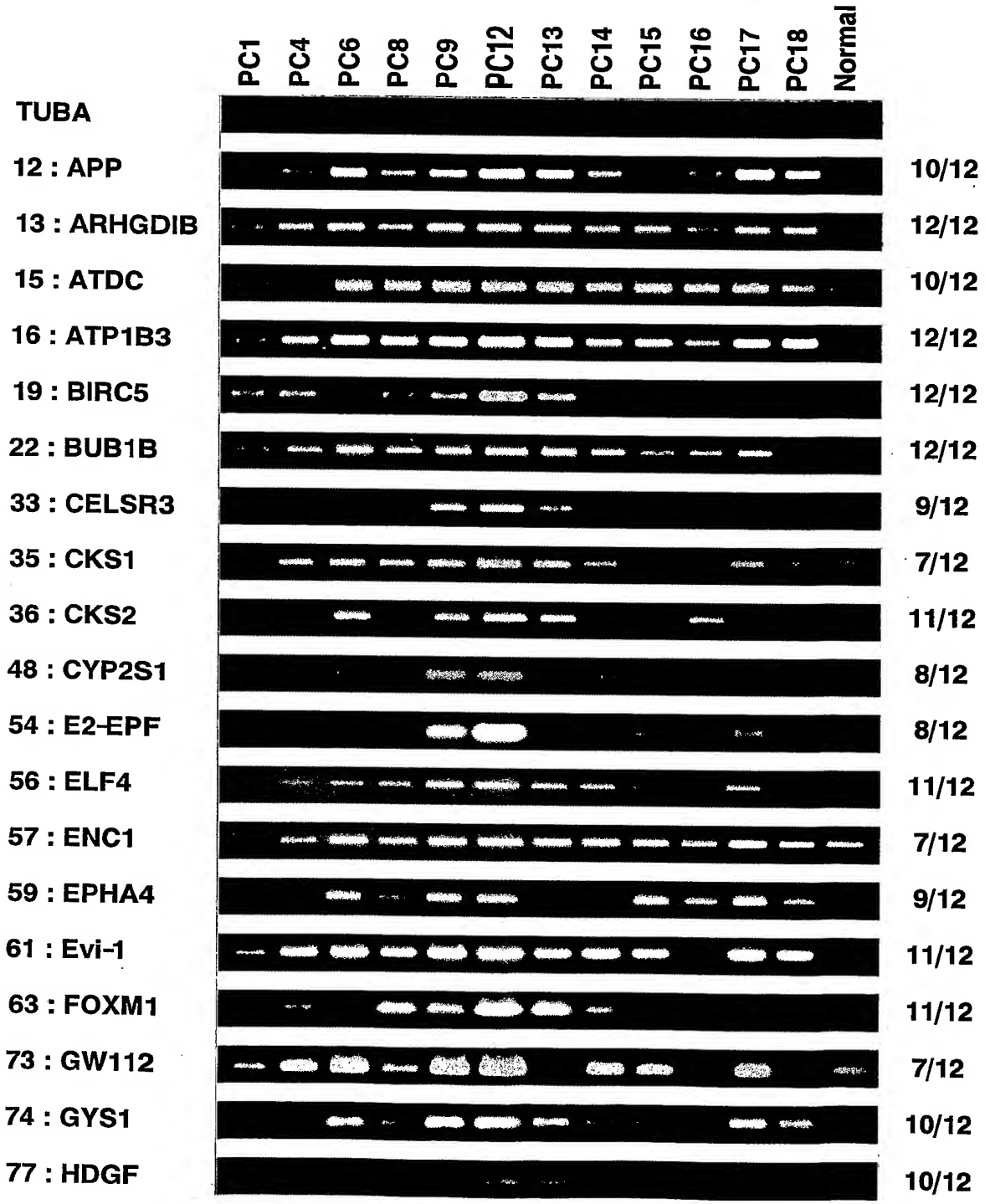


FIG.2-1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PC6
PC8
PC9
PC12
PC13
PC14
PC15
PC16
PC17
PC18
Normal
TUBA
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13 : ARHGDIB
15 : ATDC
16 : ATP1B3
19 : BIRC5
22 : BUB1B
33 : CELSR3
35 : CKS1
36 : CKS2
48 : CYP2S1
54 : E2-EPF
56 : ELF4
57 : ENC1
59 : EPHA4
61 : Evi-1
63 : FOXM1
73 : GW112
74 : GYS1
77 : HDGF
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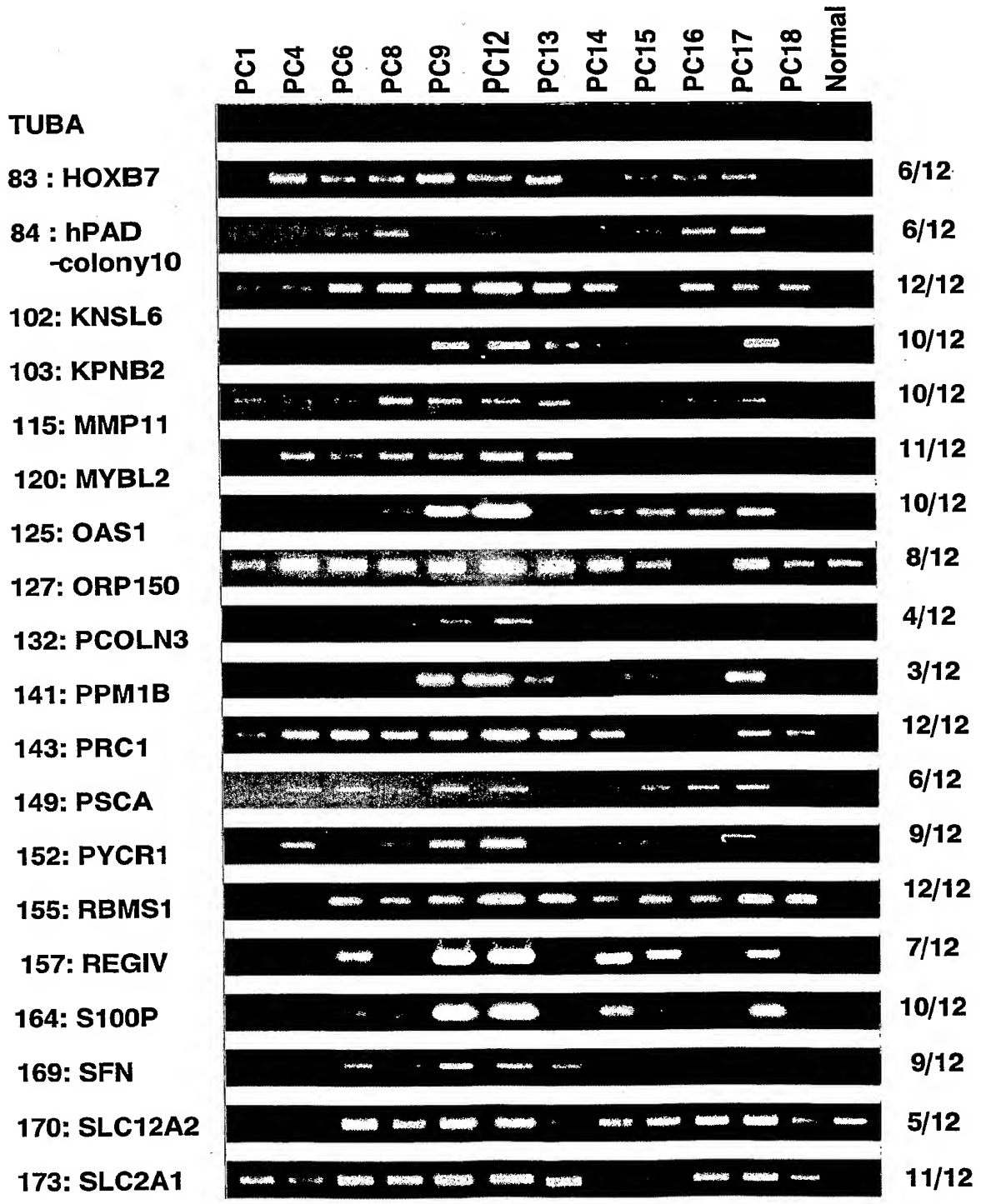
FIG.2-2


PC1
PC4
PC6
PC8
PC9
PC12
PC13
PC14
PC15
PC16
PC17
PC18
Normal
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84 : hPAD -colony10
102: KNSL6
103: KPNB2
115: MMP11
120: MYBL2
125: OAS1
127: ORP150
132: PCOLN3
141: PPM1B
143: PRC1
149: PSCA
152: PYCR1
155: RBMS1
157: REGIV
164: S100P
169: SFN
170: SLC12A2
173: SLC2A1
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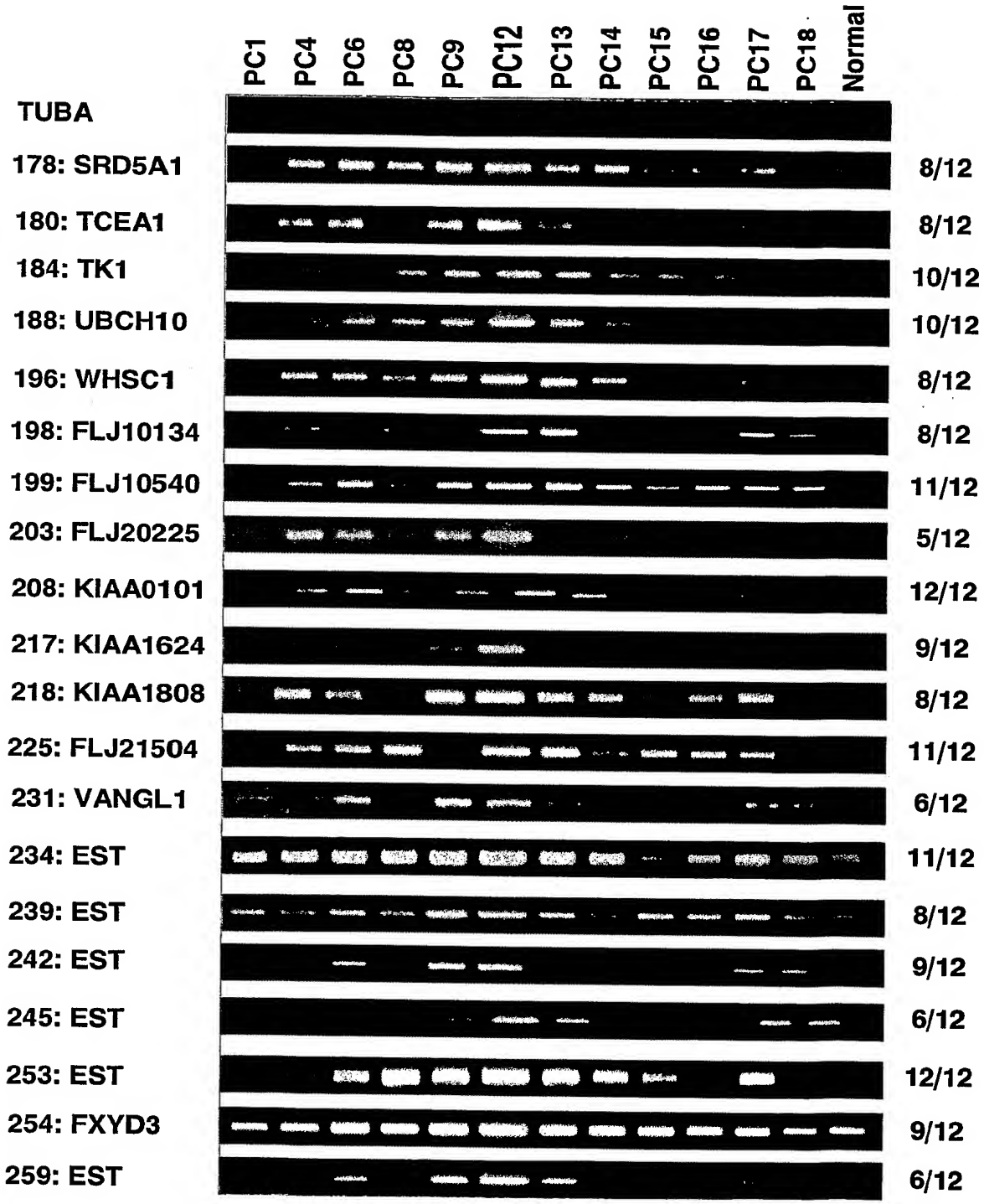
FIG.2-3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PC6
PC8
PC9
PC12
PC13
PC14
PC15
PC16
PC17
PC18
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180: TCEA1
184: TK1
188: UBCH10
196: WHSC1
198: FLJ10134
199: FLJ10540
203: FLJ20225
208: KIAA0101
217: KIAA1624
218: KIAA1808
225: FLJ21504
231: VANGL1
234: EST
239: EST
242: EST
245: EST
253: EST
254: FXYD3
259: EST
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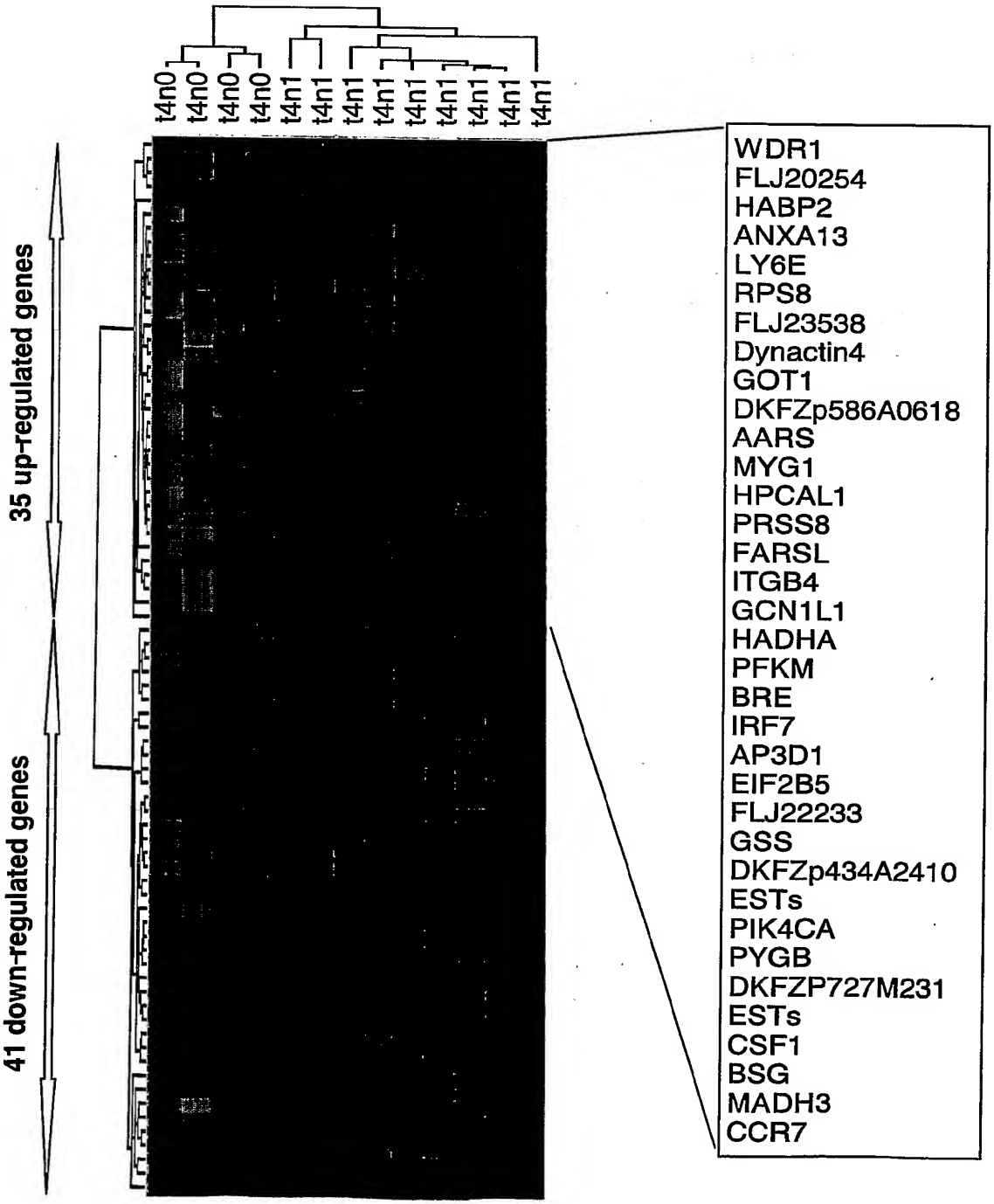
FIG.3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t4n1
35 up-regulated genes
41 down-regulated genes
WDR1
FLJ20254
HABP2
ANXA13
LY6E
RPS8
FLJ23538
Dynactin4
GOT1
DKFZp586A0618
AARS
MYG1
HPCAL1
PRSS8
FARSL
ITGB4
GCN1L1
HADHA
PFKM
BRE
IRF7
AP3D1
EIF2B5
FLJ22233
GSS
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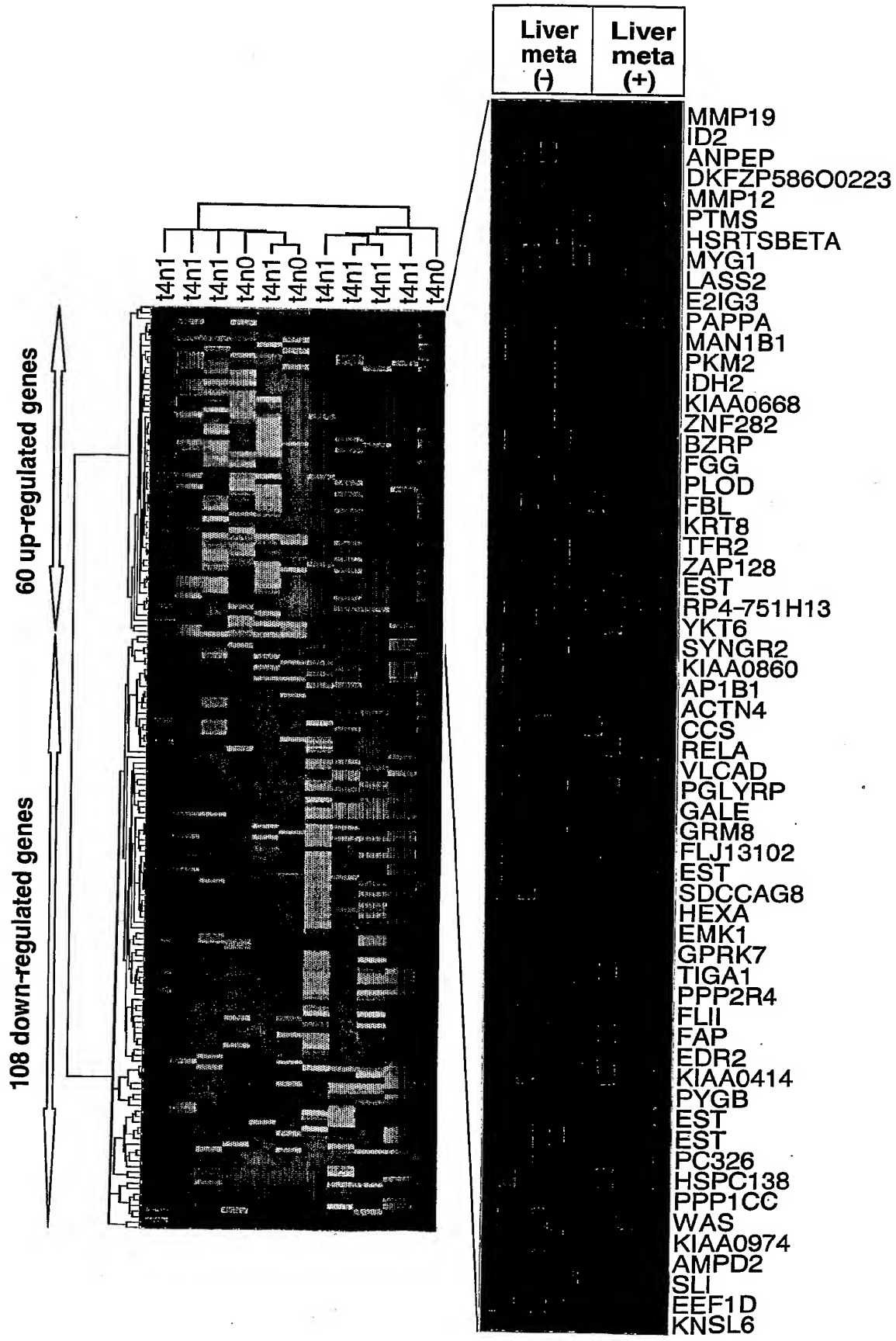
ESTs
CSF1
BSG
MADH3
CCR7

FIG.4


Liver meta (-)
Liver meta (+)
t4n1
t4n1
t4n1
t4n0
t4n1
t4n0
t4n1
t4n1
t4n1
t4n1
t4n0
60 up-regulated genes
108 down-regulated genes
MMP19
ID2
ANPEP
DKFZP586O0223
MMP12
PTMS
HSRTSBETA
MYG1
LASS2
E2IG3
PAPPA
MAN1B1
PKM2
IDH2
KIAA0668
ZNF282
BZRP
FGG
PLOD
FBL
KRT8
TFR2
ZAP128
EST
RP4-751H13
YKT6
SYNGR2
KIAA0860
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SDCCAG8
HEXA
EMK1
GPRK7
TIGA1
PPP2R4
FLII
FAP
EDR2
KIAA0414
PYGB
EST
EST
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HSPC138
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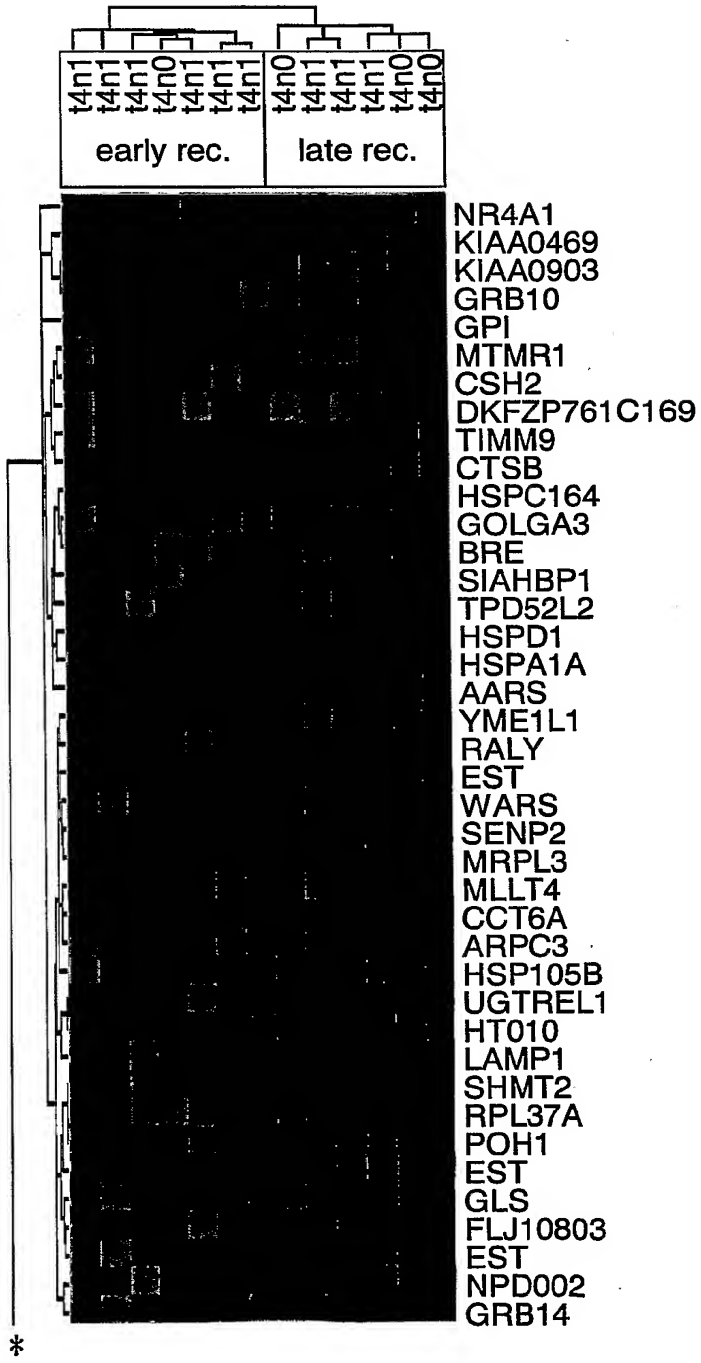
SLI
EEF1D
KNSL6

FIG.5

(A-1)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t4n1
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t4n1
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t4n1
t4n1
t4n1
t4n0
t4n0
early rec.
late rec.
NR4A1
KIAA0469
KIAA0903
GRB10
GPI
MTMR1
CSH2
DKFZP761C169
TIMM9
CTSB
HSPC164
GOLGA3
BRE
SIAHBP1
TPD52L2
HSPD1
HSPA1A
AARS
YME1L1
RALY
EST
WARS
SENP2
MRPL3
MLLT4
CCT6A
ARPC3
HSP105B
UGTREL1
HT010
LAMP1
SHMT2
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FLJ10803
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NPD002
GRB14
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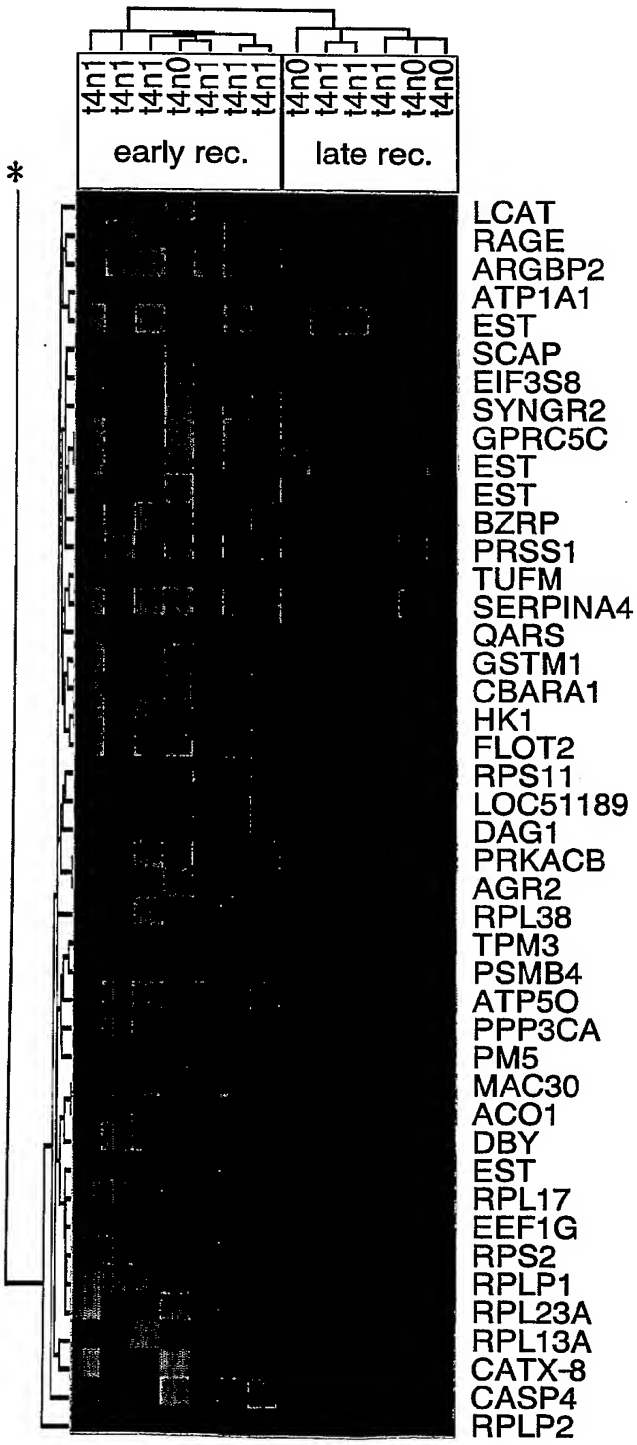
FIG.5

(A-2)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t4n1
t4n1
t4n0
t4n1
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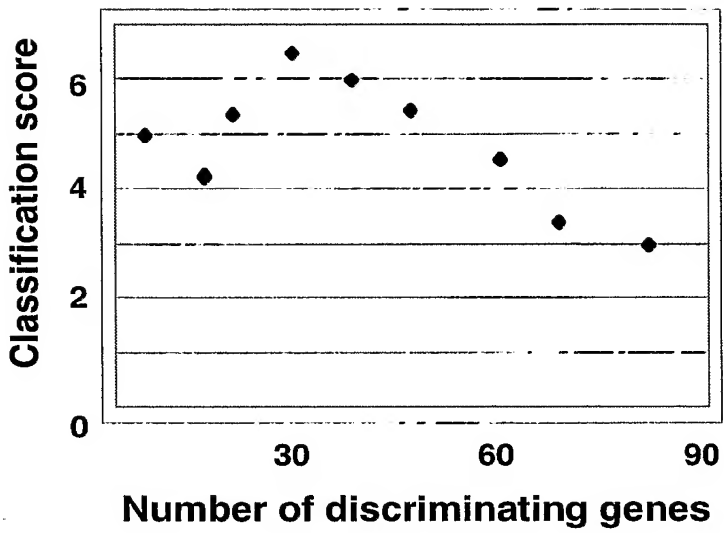
t4n1
t4n0
t4n1
t4n1
t4n0
t4n0
early rec.
late rec.
*
LCAT
RAGE
ARGBP2
ATP1A1
EST
SCAP
EIF3S8
SYNGR2
GPRC5C
EST
EST
BZRP
PRSS1
TUFM
SERPINA4
QARS
GSTM1
CBARA1
HK1
FLOT2
RPS11
LOC51189
DAG1
PRKACB
AGR2
RPL38
TPM3
PSMB4
ATP5O
PPP3CA
PM5
MAC30
ACO1
DBY
EST
RPL17
EEF1G
RPS2
RPLP1
RPL23A
RPL13A
CATX-8
CASP4
RPLP2

FIG.5

(B)


Classification score
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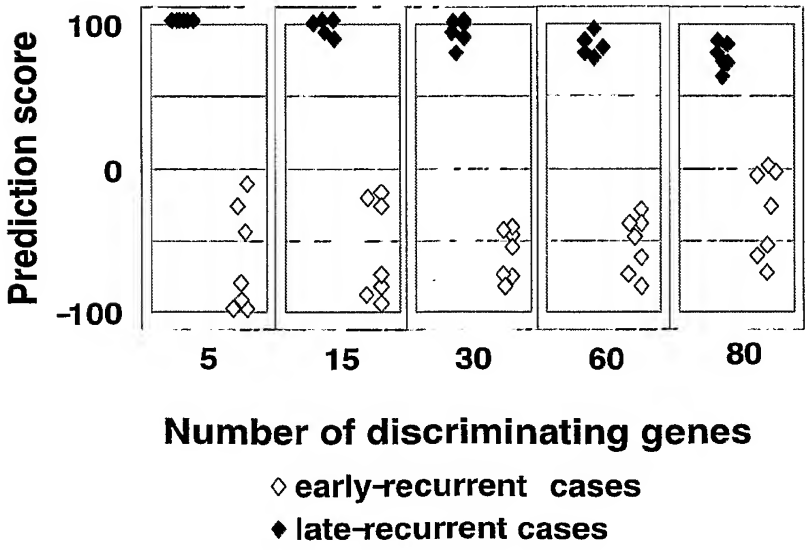
4
2
0
30
60
90
Number of discriminating genes

(C)


Prediction score
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-100
5
15
30
60
80
Number of discriminating genes
◇ early-recurrent cases
◆ late-recurrent cases

SEQUENCE LISTING

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JAPAN AS REPRESENTED BY THE PRESIDENT OF THE UNIVERSITY OF TOKYO

<120> METHOD FOR DIAGNOSING PANCREATIC CANCER

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<151> 2002-09-30

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PCT REQUEST

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VIII-3-1	**Declaration: Entitlement to claim priority** Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application specified below, where the applicant is not the applicant who filed the earlier application or where the applicant's name has changed since the filing of the earlier application (Rules 4.17(iii) and 51bis.1(a)(iii)): Name:	in relation to this international application ONCOTHERAPY SCIENCE, INC. is entitled to claim priority of earlier application No. 60/414,872 by virtue of the following:
VIII-3-1 (iv)		an assignment from NAKAMURA, Yusuke to ONCOTHERAPY SCIENCE, INC., dated 30 September 2002 (30.09.2002)
VIII-3-1 (iv)		an assignment from KATAGIRI, Toyomasa to ONCOTHERAPY SCIENCE, INC., dated 30 September 2002 (30.09.2002)
VIII-3-1 (ix)	This declaration is made for the purposes of:	AP: (GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW); EA: (AM AZ BY KG KZ MD RU TJ TM); EP: (AT BE BG CH&LI CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT RO SE SI SK TR); OA: (BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG); AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG UZ VC VN ~~YU~~ ZA ZM ZW CS

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VIII-3-2	**Declaration: Entitlement to claim priority** Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application specified below, where the applicant is not the applicant who filed the earlier application or where the applicant's name has changed since the filing of the earlier application (Rules 4.17(iii) and 51bis.1(a)(iii)): Name:	in relation to this international application ONCOTHERAPY SCIENCE, INC. is entitled to claim priority of earlier application No. 60/450,889 by virtue of the following:
VIII-3-2 (iv)		an assignment from NAKAMURA, Yusuke to ONCOTHERAPY SCIENCE, INC., dated 31 January 2003 (31.01.2003)
VIII-3-2 (iv)		an assignment from KATAGIRI, Toyomasa to ONCOTHERAPY SCIENCE, INC., dated 31 January 2003 (31.01.2003)
VIII-3-2 (ix)	This declaration is made for the purposes of:	AP: (GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW); EA: (AM AZ BY KG KZ MD RU TJ TM); EP: (AT BE BG CH&LI CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT RO SE SI SK TR); OA: (BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG); AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG UZ VC VN ~~YU~~ ZA ZM ZW CS

PCT REQUEST

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VIII-3-3	**Declaration: Entitlement to claim priority** Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application specified below, where the applicant is not the applicant who filed the earlier application or where the applicant's name has changed since the filing of the earlier application (Rules 4.17(iii) and 51bis.1(a)(iii)): Name:	in relation to this international application JAPAN AS REPRESENTED BY THE PRESIDENT OF THE UNIVERSITY OF TOKYO is entitled to claim priority of earlier application No. 60/414,872 by virtue of the following:
VIII-3-3 (iv)		an assignment from NAKAMURA, Yusuke to JAPAN AS REPRESENTED BY THE PRESIDENT OF THE UNIVERSITY OF TOKYO, dated 30 September 2002 (30.09.2002)
VIII-3-3 (iv)		an assignment from KATAGIRI, Toyomasa to JAPAN AS REPRESENTED BY THE PRESIDENT OF THE UNIVERSITY OF TOKYO, dated 30 September 2002 (30.09.2002)
VIII-3-3 (ix)	This declaration is made for the purposes of:	AP: (GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW); EA: (AM AZ BY KG KZ MD RU TJ TM); EP: (AT BE BG CH&LI CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT RO SE SI SK TR); OA: (BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG); AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG UZ VC VN ~~YU~~ ZA ZM ZW CS